

Democracy's day

THE House of Commons last Wednesday took its first opportunity to debate the Windscale issue—whether Britain should expand its facility for reprocessing nuclear fuel at Windscale, Cumbria, to a plant capable of taking 1,200 tonnes of oxide fuel per year, and whether it should sign contracts with foreign countries (particularly Japan) to reprocess their fuel. For six and a half hours a few MPs, some well-informed, some not, tossed the issues back and forth; but to what avail?

Three government ministers intermittently attended the debate, to give it credence: Mr Peter Shore, Secretary of State for the Environment, who opened for the government; Mr Anthony Wedgwood Benn, Minister of State for Energy, who looked serious but said nothing; and Dr David Owen, Minister of Foreign and Commonwealth Affairs, who protested his concern for controlling the proliferation of nuclear weapons, and argued that THORP (the proposed facility) would reduce proliferation risks. Mr Shore was there longest, as the Minister responsible for the 100-day public inquiry into the THORP proposal (the Windscale inquiry), and he lounged on the front bench seemingly disdainful of the games the other MPs were playing around him. Occasionally he rose to point out to the children where they were going wrong. The division (at 11 pm) went 186 to 56 for THORP and everyone went home. Democracy had had its day.

It is frightening that matters of such consequence can be dealt with so peremptorily. It is a great disservice to those few MPs who understand the full seriousness of the issue that others reduce their case to one of being pro- or anti-nuclear. The few need an effective voice. We have made it clear in these pages that the essential political issues in the THORP application are blatantly prejudged in Mr Justice Parker's report on the Windscale inquiry, and for that reason it is specious for Ministers to argue that parliament is not being "hustled into a decision" as Mr Leo Abse put it. We need more time—and the place—for thorough political analysis.

Whether THORP will increase or decrease the risk of the proliferation of nuclear weapons is a finely balanced issue on which everyone—including *Nature*—has a view. Dr David Owen last Wednesday expressed the view of the British Government that THORP will decrease risks. That is, of course, whatever its merits, a very convenient view, given that THORP according to its designers' estimates might ultimately provide Britain with £600 million of foreign exchange. The US, sitting on one third of the world's uranium reserves (and so not under great pressure to conserve uranium), and with reactors using fuel cans that can be stored in cooling ponds for at least a decade, takes the reverse view. Mr

Robin Cook, MP for Edinburgh Central, told the House last week what the US Senate Subcommittee on energy and nuclear proliferation thinks about our reprocessing plans. In a letter to President Carter dated 8 March, Senator John Glenn and four colleagues wrote about US non-proliferation policy, which has called a halt to reprocessing: "An expansion of existing European reprocessing plants could shatter our attempts to forge a consensus on less destabilising alternatives. . . . To grant reprocessing approvals for fuel of US origin on a scale necessary for keeping the enterprise alive would be to make a mockery of our policy". It is clearly possible to have more than one view on the dangers of proliferation arising from reprocessing. Can MPs reach a balanced and informed position on this issue in six and half hours of debate? They certainly did not do so last Wednesday.

Equally vexed and complex are the issues surrounding the use of THORP as a means of dealing with nuclear waste. More than one MP pointed out in the debate that Mr Justice Parker had recognised that the vitrification of high-level (fission-product and actinide) waste, and its ultimate geological disposal, were unproven but had insisted that these technologies must be developed. Why then had he not insisted that the technology of dry storage of untreated fuel, as proposed by Friends of the Earth, be developed also? What exactly are the merits of the case? How can MPs resolve that?

We argued last week that there are certain points in the reprocessing issue that are essentially political and so must be resolved by political process. These are energy futures (not a matter of plotting graphs); waste management; and proliferation.

In addressing these issues as political, one might have thought of parliament, the centre of democracy in the UK, as being the place to resolve them. And so it should be. But not a parliament where the bulk of MPs are ill-informed, and where the debate staggers patchily from one tired point to another, until the occasional member who has truly studied the case gets a chance to speak. We need a democratic forum where MPs informed and interested in the issues can have a long and thorough discussion in depth; where they can call witnesses; where they can call on a modicum of research staff; and where they can produce reports which will be read and respected, instead of producing speeches lost over the heads of reclining ministers. The Select Committee of Science and Technology is too busy with other affairs; and its chairman is well known for his partisan approach to energy matters. Last week's debate may now be lost in the sands of *Hansard*; but it taught us one thing: that energy now deserves its own Select Committee. □



The moralistic fallacy

Bernard B. Davis of the Bacterial Physiology Unit, Harvard Medical School, discusses whether or not scientific inquiry should be blocked on moral grounds

THE increased focus of our age on social justice, and on the need to control the costs of technology, has had admirable consequences. But it has also reactivated an old threat to science: the demand that certain kinds of scientific knowledge be forbidden. George Steiner, in his recent Bronowski Memorial Lecture, rejected this proposal, on the pragmatic grounds that it just won't work. However, *Nature's* editorial of 2 February, drew a different conclusion: that we are now groping for a code to protect society from dangerous knowledge, much as we have developed ethical codes to protect the subjects of biomedical research.

Since this issue is of central importance for the future of science we must consider the arguments very carefully. I wish to discuss some that did not appear in Steiner's lecture or in *Nature's* editorial. I shall focus on the heritability of intelligence, which Steiner views as the most intractable among the several kinds of dangerous scientific knowledge.

First, the analogy to medical research proposed in the editorial, though plausible at first glance, in fact ignores a crucial distinction: between actions that are themselves dangerous and knowledge that might lead to dangerous actions. Medical investigators do indeed accept ethical limitations on dangerous procedures, that is, those that would expose an experimental subject to loss of dignity, pain, or risk. And investigators in behavioural genetics are subject to analogous limitations: they cannot mate humans at will, or transfer identical twins into different homes, even though these procedures would be powerful tools for advancing knowledge. On the other hand, medical investigators are not forbidden to seek knowledge simply because it may be painful, in its prognostic implications, for the subject or for others. So medicine does not provide a model for justifying limitations on the knowledge sought in other areas.

The very concept of dangerous knowledge is also shaky. Ever since the discovery of fire, and of cutting tools, it has been clear that virtually any scientific knowledge can be used for good or for ill: the costs and benefits depend entirely on how it is used. Moreover, we have only a very limited ability to foresee the eventual scientific benefits of a new discovery: science is a continuous web, and fundamental advances often arise through unexpected cross-fertilisation. For example, there are very good reasons to forbid human cloning: but if we should forbid any research in cell biology that might bring cloning nearer we would seriously impair advances in cancer research. We must therefore ask whether it is more rational to try to protect society by limiting the areas open to fundamental inquiry, or by focusing on earlier assessment and improved control of new technological applications of scientific knowledge.

We must also consider the rather ahistoric and absolutist conception of justice implied in the suggestion of a fundamental incompatibility between man's hopes of justice and decency and certain categories of truth. For though it is clear that the concept of justice has certain stable features, it is also clear that the rules of behaviour in any society, and the assumption underlying these rules, are continually evolving—especially when the society is faced with new knowledge and new technologies. For example, we have weathered the storm created by Darwin; and though the supernatural basis for a moral consensus was shattered by his elimination of special creation, we have meanwhile developed a radical increase in our sensitivity to problems of human rights. Can we not trust posterity also to adapt

its notions of morality to further new knowledge?

More specifically, there seems to be a pervasive fear of the social impact of genetics, arising largely from the pseudoscientific extrapolations of social Darwinism and Nazi racism. I would suggest that this view does not reflect the real contributions of this field. In fact, one of the historical grounds for racism was the pre-scientific conception of races as permanent, distinct products of creation. But evolution made us aware of the brotherhood of all races. Another rationale for racism was the typological conception of the nature of race—a view based on the Platonic characterisation of groups in terms of an ideal "type", subject to only minor deviations in concrete individuals. But this misconception was destroyed by population genetics, which demonstrated the great genetic heterogeneity of all races, the statistical nature of the differences between them, and the extensive overlap of these distributions for most traits. This field has thus contributed far more than is recognised to public awareness that one cannot determine an individual's capacities by identifying him with an ethnic group.

Finally, we must ask how much the current reasons for proscribing an area of knowledge really differ from those used by Urban VIII, Bishop Wilberforce, or Lysenko. The main difference is that science is penetrating increasingly into areas that generate moral problems, and that generate technological capacities for great destruction. Since Steiner considered the former the more intractable, we might look more closely at his suggestion that a demonstration of heritable differences in the distribution of abilities among different ethnic groups might be irreconcilable with human justice. Apart from the implication of a fixed rather than an adaptive concept of justice, noted above, this proposition seems to be blaming the messenger for the message. For science does not create the realities of nature: it only discovers them. And if it is not allowed to discover them they will still be there, determining whether or not our assumptions and our predictions turn out to be correct.

Recognition of the distinction between reality and the knowledge of reality has profound consequences. It tells us that if we wish to build social policy soundly we must not confuse the normative with the empirical. More specifically, we must rest the goal of racial justice on grounds of moral conviction, rather than on vulnerable assumptions about questions of fact; and we must recognise that we can adapt our social institutions to our evolutionary legacy, but not *vice versa*. We must also recognise that justice and equality are subtle and complex concepts however simplistic the forms that they assume in the ideological marketplace: and these concepts will eventually have to be defined in ways that do not depend on a particular assumed distribution of abilities. If we choose otherwise, and suppress human behavioural genetics for fear that the results may contradict our assumptions, the costs may be high. For a major goal of this field, long emphasised by J. B. S. Haldane, is to help us to adjust educational procedures to individual differences in cognitive potentials and in patterns of learning.

For several reasons, then, the assumption of an inherent conflict between genetics (or other areas of science) and justice seems philosophically unsound. The objections can be summarised quite simply: since blocking off an area of inquiry on moral grounds fixes our knowledge in that area, it becomes, in effect, an illogical effort to derive an 'is' from an 'ought'. I would suggest that we call this procedure the moralistic fallacy, since it is the mirror image of what Hume and G. E. Moore identified as the naturalistic fallacy. But, alas, identification may not get us very far. For as Stephen Toulmin recently emphasised in *Daedalus*, we are in the midst of one of history's swings between a romantic concern with the good and a classic concern with truth. □

Health and safety in research laboratories

Fred Grover and Peter Wallace discuss some of the problems of applying the UK's Health and Safety at Work Act to workers in research laboratories

THE UK Health and Safety at Work Act, 1974 differs from previous legislation in one major respect: it applies to all persons at work and, therefore, to many people not covered by earlier legislation. Laboratory workers are among these 'new entrants' and although previous regulations have applied in part to their work there has never before been legislation which is relevant to all their activities. Inspectors appointed under the Act have only recently begun to examine those laboratories not previously visited and in due course they will all come under scrutiny. It is interesting to wonder how the inspectors' findings will compare with those in industry. Will present standards be acceptable to them?

Common room conversations reveal that a large proportion of laboratory workers think that safety standards are already adequate and that inspectors would find little cause for complaint. It is true that most well run laboratories have been safety conscious for many years and that accident rates have been relatively low; but this happy state of affairs has often been the result of bitter experience. Any hint of complacency is a danger signal: to rely on past record rather than future improvement can only lead to declining standards and ultimate catastrophe.

The problem with the Act is that it is at once precise and vague; precise in that it defines the duties of all employees and employers and vague in the way it does not stipulate what is considered to be a safe approach to any specific task. It will doubtless result in the proliferation of more specific regulations in the light of experience and case law but for the time being its operation must depend on its interpretation by individual inspectors.

Accidents do not just happen but arise from numerous and varied causes or are brought about by several apparently unrelated factors, each of which in isolation would not have been considered hazardous.

Clearly an inspector can only assess the situation from what he sees or from what is reported to him; he has no way of determining the competence, or otherwise, of individual workers. Presumably, he will be primarily concerned with the working conditions and with the safety policy of the laboratory. His experience will enable him to make a fairly accurate assessment based on a general impression gained from inspection of the laboratories, discussions with staff and examination of accident records and safety committee reports. One assumes also that inspectors will, where necessary, call in specialist advisers to give guidance on safe working methods and that in time the

majority of foreseeable hazards can be virtually eliminated.

Thorough inspection of the premises and working methods, however, may not reveal any significant defects; but this does not guarantee that accidents will not occur. The provision of fire extinguishers does not prevent a fire from starting and the presence of an autoclave in a bacteriology department does not prevent the escape of pathogenic organisms.

What then can be done about the less obvious but equally significant behavioural problems which cannot be overcome either by legislation or inspection? What evidence is there to support the view that laboratory staff are inherently safer than other workers? To assume that a higher level of education is a password to safety is not only turning a blind eye to the problem but may well be a hazard in itself. Immunity from accidents is not a bonus issued free with every degree or certificate of technical ability. Carelessness, fatigue, lack of concentration, shortage of time, even bravado are just some of the possible causes of accidents but perhaps the dominant factor is simply a lack of common sense.

Experience and training

Common sense is not a subject which can be taught academically but is acquired through experience and training. The young employee, entering the laboratory environment for the first time, is potentially more at risk than his experienced colleagues and will require more supervision and guidance. A very junior technician was badly burned with sulphuric acid which he was pouring from a winchester into a small test tube, holding the bottle in one hand and the tube in the other. He had simply not realised the impossibility of doing this safely.

The more experienced worker coming from an organisation where safety is not taken seriously can bring problems with him. He will probably have survived for years with no major mishap and will not take kindly to working within a new set of rules. Academic freedom is, of course, essential to scientific progress but like every other freedom it must be limited by a consideration for the rest of society.

A biochemist, having been advised of the dangers of perchloric acid at high temperatures, insisted that no other oxidant would be suitable for



General view of an analytical laboratory

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his particular need and that what he was about to do was a standard technique. The reagent was added to a number of tubes in an aluminium heating block and within minutes the resulting explosion removed all the glass from the fume cupboard in which he was working.

Changing techniques can lead an experienced worker into areas totally new to him. A parasitologist of many years experience became involved in a technique using ^{125}I which he counted on planchettes as a solid iodinated protein. He soon discovered that the protein would not remain in the planchettes when they were handled and was advised to add an adhesive to fix the material. This idea did not appeal to him and he placed the planchettes on the bench and sprayed them with an aerosol of microscope slide fixative which he had been in the habit of using in his normal work. Within seconds he had emptied the planchettes and contaminated his laboratory.

Some years ago the need for specialisation was not so apparent as it is today and both education and training were of a more general nature. Basic skills which were once considered so essential now tend to be overlooked or regarded as the province of some other discipline. A pathologist, having received limited training in chemistry, may be totally unaware that hypochlorites and formaldehyde react, possibly even in the vapour state, to form Bis-chloromethyl ether, a potent carcinogen.

It used to be customary to refer to a chemist but it is now fashionable to think in terms of a plastics chemist, a pesticide chemist or a paint chemist.

Each of these may know nothing of the others' technology or of the associated hazards. A virologist and a parasitologist jointly became involved in a new technique which required the addition of sodium azide to several hundred test samples. After some months it was discovered that they had routinely disposed of their residues in the sink, totally unaware of the explosive properties of azides.

The worker whose time is in great demand is often under such pressure that he becomes forgetful. A biochemist needing distilled water of a higher purity than that provided by the departmental still, set up a glass still over a gas burner and rushed off to a meeting. In his absence the flask cracked, extinguishing the burner and filling the room with gas.

The external standard source of a liquid scintillation counter became wedged in the pneumatic transfer system and a workshop technician, familiar with the equipment, was called in to clear the fault. Complete dismantling would have taken some

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Testing for mutagens

considerable time but having been told that results were needed urgently he attempted a quick cure by disconnecting the flexible tube and blowing into it, without success. As he released the pressure the radioactive source blew back into his mouth. Fortunately he did not swallow it.

Health as well as safety

Discussions and publications relating to the Act have laid great emphasis on the safety aspect but health and welfare which also featured in the original document have received little attention. There are countless laboratory situations which may not result in accidents but which are decidedly unhealthy. Inadequate ventilation may result in prolonged exposure to low levels of toxic vapours which might produce clinical symptoms in later life. The long term carcinogenic properties of various groups of compounds are still something of a mystery but it is abundantly clear that risk is related to concentration and period of exposure. Many occupational diseases of the past were simply not associated with exposure to toxic materials and as the list of such compounds grows it becomes apparent that we are not yet out of the wood.

The whole problem of bio-hazards is still somewhat nebulous (there is apparently no legal definition of the word) and it is only recently that any serious attempt has been made to distinguish between dangerous and very dangerous pathogens. Labelling an organism low risk as opposed to high risk does not imply that it is safe to handle under all conditions, an assumption that may well be made by the inexperienced.

There are many examples of major and minor accidents and of near-misses which add to an awareness of the shortcomings. Management has a clearly defined obligation under the Act to provide conditions and training to ensure safety "so far as is reasonably practicable" (a phrase which occurs no less than fourteen times in the first six sections).

A detailed examination of laboratory surroundings is clearly a good starting point and the rectification of obvious weaknesses may well satisfy the inspectors. But this, is itself, is not sufficient: health and safety are a philosophy and every available means must be used to instill correct attitudes among staff at all levels. The Act refers to employers and employees but in many respects senior staff may be regarded as either or both.

Good housekeeping, training, supervision and discipline are all vital but constant vigilance is probably that all important factor in reducing accident levels. The cost of accidents is too great, both in material terms and in terms of human suffering, to allow for complacency.

What once may have been considered as little more than a moral obligation is now a legal commitment. This year, many organisations are to introduce Safety Committees whose terms of reference and *modus operandi* must be clearly defined and agreed. In some establishments this is regarded as another piece of time wasting bureaucracy and problems will inevitably arise. The Health and Safety at Work Act is a genuine attempt to reduce the awesome toll of death and injury which for too long has gone unchecked. □

West German model predicts low growth in energy demand

ACCORDING to a recent study of the Institute for Applied Systems Research and Prognosis (ISP), West Germany is building nuclear power stations too quickly. The study estimates that by 1985 only 16,000 MW of nuclear energy will be needed in contrast to the 25,000 MW now in operation or under construction. (Only a few years ago, an official estimate put the figure at 45,000 MW).

The ISP was founded in 1975 by Professor Eduard Pestel. It evolved out of the work for *Mankind at the Turning Point*, the second report of the Club of Rome, in which most of its present members participated. The results of its German study or *Deutschland modell* will be published in April. The German government, has already seen them but has not yet reacted: only the nuclear industry has shown any interest so far. At a recent gathering of European science journalists in Hannover, however, Professor Pestel and his team made their results public.

Professor Pestel commented that the national model—embedded in the world model of Forrester and Meadows—that he and his team had built up can be used as a tool for formulating many facets of national policy. In particular, the study deals with changes in population, the changing educational system, the development of the national economy within the world economy and the demands for energy, resources, capital and labour. The specific problems of electrical generating capacity and unemployment are also considered.

The study foresees an average growth in gross national product (GNP) of

2.4% until 1984. After that growth is expected to slow down to below 2% by 1990 and then to pick up to 2.2% after 1992. The allocation of the GNP however, will remain roughly the same as now. Investments are expected to increase in the early 1980s.

A steady decline in population from the maximum of 62 million in 1972 to 56 million in 2000 is expected. Unemployment will increase during the next decade. The number of unemployed is expected to rise to two million by 1982 and to reach a maximum of four million by 1990. A major factor affecting unemployment is seen as the mismatching between qualified personnel and the types of employment available. Different scenarios are suggested to avoid constant unemployment, one of

them being a combination of three more days vacation, a reduction of the weekly working time by two hours and a 5% increase in part-time jobs.

The ISP study estimates that Germany's total primary energy input will increase from 370 million tons of coal equivalent (TCE) now to 437 million TCE in 1985. In 1973, the German government forecast for 1985 was 610 million TCE; in 1977 it was 497 million TCE. The ISP expects crude oil to remain the most important primary energy source.

In contrast to many other studies, the ISP one does not foresee a gap between energy supply and demand from 1985 to 1990. The greatest difference, however, between the ISP and government forecasts is for nuclear power. The ISP model estimates that nuclear power will be needed to generate 37 million TCE in future whereas the government estimates that 62 million TCE will have to be nuclear.

Casper Schuurin

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Twin towers of the nuclear plant at Wurgassen in West Germany

Ecologists lose in French elections

AMONG the losers in the French parliamentary election last week are the representatives of the various ecological groups that had joined forces under the label of *Collectif Ecologie '78*. They represent people from left and right, labourers and executives, students and scientists, who opposed civil and military nuclear programmes, urged energy saving measures and the development of new sources of energy, environmental-impact studies for public projects, and in general the re-evaluation of the goals of material growth upheld by all of the traditional parties.

Les Verts (the Greens), one of the ecological groups, polled a surprising

5% of the votes in a municipal election last year. A comparable success in the first turn of the parliamentary elections would have given them considerable political leverage, particularly if the votes could be passed on the second turn to candidates of another party.

But the ecologists had agreed not to recommend any other party candidates on the second turn because they did not want to be identified with any traditional party. The parliamentary election, however, was a much more decisive one than the municipal election and this is probably why Frenchmen voted for more business-like candidates. The Greens received only 2.2% of the total votes. They then made a mis-

take that may be held against them in the future; as soon as the results of the first turn were announced, Brice Lalonde, one of the principle spokesmen for *Collectif Ecologie '78* urged that ecologists cast their ballots for the left-wing opposition on the second turn. Other Greens protested against this breaking of the pre-electoral pledge, and the '*Collectif Ecologie*' appeared to dissolve.

On the outcome of the final vote the Greens had, as expected, no deputies and no-one knows whether they contributed to the left or the right.

The early hopes of the movement have been shattered and its future is now dubious.

Alexandre Dorozynski

Medical profession to investigate torture

"TORTURE should become a field of scientific research, to include forensic study, the training of torturers, the detection of torture marks, and, ultimately, the provision of improved medical care for torture victims."

Such was the startling conclusion of more than 100 doctors from Europe and North and South America, attending a seminar on "Violations of human rights: torture and the medical profession," held in Athens on 10-11 March. The doctors see the results and incidence of torture as "a traumatic disease—inflicted by humans on humans—that should be investigated and publicised by the medical profession."

This "disease", although endemic in human society, has of recent years reached almost epidemic proportions, while there are an increasing number of allegations of the involvement of doctors in such practices, either by using their knowledge to design and

"improve" torture techniques, or by acting as observers, to ensure that the victim does not succumb prematurely. This has caused considerable alarm within the medical profession, and there have been several small meetings to discuss what action the profession as a whole should take. The Athens seminar was the first major gathering.

In addition to advocating research into the sociological and psychological factors involved in the problem of torture, the assembled doctors recommended that governments and inter-governmental organisations, especially the UN, should consider new means of helping torture victims, and also that an international convention should be drafted, which would clarify the responsibility of the state for the financial compensation and rehabilitation of torture victims. (At present, many victims are unable to claim any aid, on the grounds that the present regime

was not responsible.)

The doctors' stand is seen by human rights campaigners as analogous to that of the psychiatrists against the political misuse of psychiatry for political repression—a campaign which culminated in the resolution of the World Psychiatric Association (Honolulu, 1977) censuring the psychiatric repression of dissidents in the Soviet Union. This campaign, of course, is by no means over—although the latest reports from Moscow indicate that the censure motion has caused a gallant handful of Soviet psychiatrists to opt out of such political "treatments". At least seven are known to be suffering reprisals for this refusal, two of whom, Drs Olga Viktorovna Makarova and Anatolii Nikitich Barabanov have been arrested. According to one report, Dr Barabanov has now himself been declared mentally ill and is undergoing compulsory "treatment" in a penal psychiatric hospital.

Vera Rich

More Comecon cosmonauts

FOLLOWING the successful and record-breaking mission of Salyut-6/Soyuz-26/27-28, plans are now firmly underway for the next two Comecon cosmonauts each with a Russian companion, to go into orbit "before the end of the year". According to Aleksei Leonov, Deputy Head of the Soviet Space Training Centre, they will be a Pole, then an East German. Representatives of all the other Comecon countries (including Cuba and Mongolia) have now arrived at the Gagarin Space Centre, to begin training for the flights that "before 1983" will further proclaim the ideal of socialist cooperation in space.

The scientific, as opposed to the symbolic, value of such participation will clearly vary from country to country. Cuba and Mongolia, for example, contribute to the Comecon "Interkosmos" programme mainly by providing tracking facilities. It is unlikely that they will contribute much new science. Czechoslovakia, however, made considerable contribution scientifically.

Czechoslovak microbiologists provided special *Chlorella* strains, including mutations deprived of chlorophyll. Although *Chlorella* experiments had been carried out on Salyut-4 and at an earlier stage of the Salyut-6 mission, access to the "unique collection" of strains produced in Czechoslovakia made a considerable difference. For the first time, active growth was observed. In the past, the growth has been retarded in space conditions.

Czechoslovakia also provided an oxymeter, for monitoring the effect of weightlessness on oxygen in human tissue. In deference to Czech national

sentiments, the press releases stressed that this was based on Heyrovsky's principle of polarography. Similarly, the Czech capsule 'Morava', was used with the Soviet 'Splav-OI' furnace. The "Splav" experiments are concerned with production of homogeneous alloys of substances with greatly differing atomic weights, which would be impossible under conditions of gravity. They also study the mechanism of crystallisation and vitrification in weightlessness. The 'Morava' experiment extended these investigations to some electro-optical materials.

A joint experiment, with the somewhat gloomy name of 'Ekstinktsiya' studied changes in the brightness of stars setting below the earth's horizon to obtain experimental data on the presence of a layer of micrometeorite dust at a height of 80-100 km. The joint Soviet-Czechoslovak experiments were largely carried out by Remek and Gubarev his partner for Soyuz-28.

Since Salyut-6 is built to a "compartment" design, the two crews can operate independently, without seeing each other for hours on end. It is claimed that, if necessary, Salyut-6 could hold up to six people, without their getting in each other's way. Nevertheless, considerable attention is being paid to psychological stress among the crews of space stations. According to Dr Odrich Miksik of the Czechoslovak Psychiatric Research Institute, it is necessary to study how "the feeling of responsibility, time-frustration, and so on, influence decision-making in extreme situations". In the future, he said, it might be possible "to predict

the crew's responses . . . and to intervene, to make the responses as efficient as possible". This rather alarming suggestion of psychotherapy in space possibly means no more than providing sufficient rest and variety of occupation during long-term missions and of eliminating latent causes of friction. Part of the current psychological "experiment" included a special "subjective testing" scale, the "Supos-8". Work on this system began in 1967. In a Soviet experiment in 1975-6 a group of Komsomols trekked over the ice to Jan Mayen Land, recording their interpersonal relations throughout. According to Dr Miksik, the analogous Czech experiment was carried out several years earlier, using mountaineers in the Hindu Kush.

Vera Rich

Soviet scientists petition Carter

THIRTY-ONE prominent Soviet scientists have sent a letter to President Carter, calling on him to "rise above transient considerations" and renounce production of the neutron bomb. The bomb, they said, would not consolidate the power and security of the USA, because history showed that no country had yet succeeded in retaining for long the monopoly of possession of any particular weapon of mass destruction.

Signatories of the letter include Academician Anatolii P. Aleksandrov, President of the Academy of Sciences of the USSR, and the Nobel Prize-winners, Academicians N. G. Basov, L. V. Kantorovich, A. M. Prokhorov, N. N. Semenov, and P. A. Cherenkov. □

Kennedy seeks legislation on women in science

LEGISLATION establishing a ten-year programme to encourage the participation of women in scientific and technical careers has been introduced into the US Senate by Senator Edward M. Kennedy.

The proposed legislation would establish programmes, policies and procedures to enhance education for women in science and mathematics, to improve training and employment opportunities for women in science and technology, and to prevent discrimination against women in scientific and technical fields.

Particular emphasis would be placed on:

- A National Science Foundation programme aimed at interesting and involving female students in elementary and secondary school science and

mathematics courses.

- A major effort by the NSF on increasing the participation of women in courses in both higher and continuing education.

- The setting up of a clearing house on women in science, provided with an annual authorisation of \$3 million, to collect, analyse and disseminate to the public information about employment in the public and private sectors.

- Various awards to be given to those who have contributed to the advancement of women in science.

Under the proposed legislation, the head of each federal agency, national laboratory and federally-funded research and development centre is to take action to prevent discrimination against women in science and technology, to increase opportunities for

the employment and advancement of women in science and to encourage the participation of minority and physically handicapped women in science.

Introducing the bill, which would be known as the 'Women in Science and Technology Equal Opportunity Act', Senator Kennedy said that the legislation was the result of a growing concern that there had been virtually no increase in the number of women in scientific careers over the past 50 years.

"Moreover those women who are working as scientists and engineers are earning less than men in every field, at every degree level, at every level of experience and in every employment setting. Further those women who are trained in scientific and technological fields are experiencing an unemployment rate from three to five times that of men in every field of science," Senator Kennedy said.

David Dickson

YEARS ago, Glasgow was considered to be a centre of rickets, resulting from deficient diets and paucity of sunlight. Alastair Hay (24 November, page 289) mentions Glasgow as again drawing attention to rickets, this time in a recrudescence of the disease in the children of Britain's Asian community. Fifty years ago, cod liver oil was the leading prescription for preventing rickets. Swallowing it was a grim reminder of a popular adage that things that tasted bad were good for you. This old remedy has long been displaced by cheaper and more abundant vitamin D, which costs about \$20 for a million recommended daily allowances of 400 units (IU) (cod liver oil as a source of vitamin D costs 300 to 400 times as much). Why, indeed, should any child have rickets?

Most of the vitamins are promoted in overdoses to produce various forms of psychological comfort, a practice sometimes termed "acute remunerative therapy." Vitamin D is one of the two or three vitamins that are not in this category. It is not pushed in "megadoses" because its toxicity in amounts above 2,000 IU per day is well known. Except during pregnancy, there is no detectable need for additional vitamin D in most adults. These two circumstances evidently underlie the hesitation to add vitamin D to chapati flour, as reported by Hay. Much of the milk retailed in the United States, including 'non-fat' milk, contains 400 IU of added vitamin D per quart. The adoption of this procedure was a milestone in public health nutrition, comparable to

adding iodine to salt. The acceptance of such a step (immunisation is another example), depends upon benefit far outweighing risk, and upon the concept that people will participate in a measure that will help others, even though they themselves are not in need. I leave it to the sociobiologists to evaluate the matter in terms of re-

Vitamin needs



THOMAS H. JUKES

ciprocal altruism. Sometimes, however, there is a tendency for people to object to protection against a hazard with which they are no longer familiar, and which therefore is thought not to exist. This reminds one of doing away with firemen because no houses have burned down lately.

It is instructive to compare the nutritional treatment of domestic animals with that of human beings who eat them. Rickets was once a common disease in young chickens kept in poultry houses. It has long since vanished. Spurred by economics and competition, the drive to produce cheap vitamin D for poultry was a scientific success story. Chickens, and especially turkeys, could not effectively utilise the "plant" form of vitamin D, ergocalciferol, that sufficed for mammals. Ergocalciferol was made from ergosterol, present in yeast. Poultry needed the 'animal' form, cholecalciferol, that is derived from 7-dehydrocholesterol. At one time, Dupont dominated the market, which they entered in an interesting way.

First, they made a special plastic window for poultry houses that admitted ultraviolet light, which prevents rickets. Glass filters out the active wavelengths. Then they found an unusual source of 7-dehydrocholesterol in molluscs. But the organic chemists eventually outstripped the shellfish, so that vitamin D₃, cholecalciferol, became plentiful. As often happens, human nutrition became the beneficiary of research in agriculture. For this to take place, people must learn to put knowledge to use.

You may be assured that, regardless of the plight of Asian children in Great Britain, the bone of the drumstick in your fried chicken at the fast-food outlet will contain a healthy 46% to 48% of ash, on a dry, fat-free basis. It is easier to get vitamins to chickens than to children.

correspondence

Promoting renewable energy sources

SIR,—One of the many indirect lessons to be learnt from the Windscale Inquiry must be the consequence of promoting energy R&D in isolation from the wider implications arising when an energy source reaches the commercial stage.

It is generally accepted that investments now being made in possible options to oil and gas-fired power stations 20 years hence must be pitched at a high level if the options are to provide similar amounts of electricity to those at present produced by nuclear power (about 14%).

The renewable sources could form this back-up duty. However, their relatively diffuse form requires physically extensive work for the equivalent of even one large power station.

The wide and direct implications of this work could be seen by the public to be as potentially damaging in their own way as aspects of nuclear power. No time therefore must be lost in bringing forward all relevant facts for open discussion. For the renewable sources this can only be done through specific schemes in designated locations, hence studies of principles must not be divorced for long from the ways in which they may be realised in practice if the prospect of benefiting from them is not to be damagingly delayed.

Yours faithfully,

T. L. SHAW

Department of Civil Engineering,
University of Bristol, UK

Argentinian haematologist disappears

SIR,—Dr Beatriz Iparraguirre-Weinstein, Head of the Clinical Haematology Unit at Ramos Mejia Hospital, Buenos Aires, disappeared in Buenos Aires on March 8, according to a telegram I and other British haematologists have received from her husband. The telegram asks us to contact the Argentinian government at the highest levels and petition for her release. Dr Iparraguirre-Weinstein is an internationally-known expert on abnormal haemoglobins and together with my colleagues and myself the original discoverer of the important haemoglobin Buenos Aires. She is a person of the highest integrity and unlikely to be involved in any sinister political plot.

Yours faithfully,

H. LEHMANN

University Department of Biochemistry,
Cambridge

Boycotts and repressive regimes

SIR,—R. Hoffenberg's views on boycotts (2 February, page 401) are unexceptionable, if trivial. Certainly in South Africa it is not frowned on to refuse to attend a function on the

grounds of one's political views, public or private. Of course, the same might not apply to some other countries that Hoffenberg would not hesitate to visit. I am moved to point out, though, not only that the paragraph does not follow from the rest of his points, but that these are suspect at best.

It is a facile assumption that "most genuine opponents" of most "repressive" governments would have been "dealt with". Just how many million political prisoners does he think jails can hold? If the blacks who have not been 'imprisoned, silenced by banning or even murdered' are not genuine opponents, the South African government must have one of the smallest bodies of opponents in history. Hoffenberg's corollary—to let those who are not in jail suffer—is therefore arrogant.

He also seems to confuse boycotting of conferences with economic boycotts. The latter may cause hardship to people and harm to the country; the former, on the other hand, has only politically negative effects. It offers ammunition for militant isolationists, drives moderates towards the hard-liners and deprives the country of that most valuable of political leaveners: personal exposure to the views of foreign colleagues.

The cited mobility of local scientists is not nearly as valuable—what proportion of the average institution can afford an overseas trip per year? In particular, such contacts are most valuable in countries whose political systems have been formed in an atmosphere of isolation. (See also 'Saudi Arabia and the travelling scientist' 2 February, page 392).

It is hard to know what to make of the view that the gratification of political militants is adequate reason for avoiding concrete political benefits. As to the suggestion that governments make propaganda capital out of a visit, experience in South Africa contradicts it. Granting the right and need for people to take political stances, it is sad to see such an unconstructive stance urged in a journal devoted to the progress of science.

If Hoffenberg thinks that sporting boycotts achieved anything apart from enraging the local rugby and cricket fraternities, he might do worse than explain it with reference to concrete fact. In the less public sports, racial divisions owed more to social than political factors. Where there was social contact between groups, they played each other, else not. Of all the political actions that drove whites into one camp in South Africa, the sporting boycotts must have had the highest ratio of effect to cause.

More to the point, professional and scientific circles are probably the most colour-blind in South Africa. Most of those who have to modify their activities to conform to the law, usually do so under vocal protest. National or international congresses are not directly affected anyway. They are generally open to all races. Sceptics about this claim may find it illuminating to canvass the views of leading South African universities and technical societies.

Finally it seems that anyone not silenced is not worth talking to and that anyone caring enough to see for himself instead of uncritically accepting Hoffenberg's views is merely 'inquisitive'. Also, we are to dismiss out of hand those professionals who remain in the country to achieve something. If anyone feels strongly enough to want to change a country's political system, let him immigrate, work and vote there. If South Africa is your field of concern, at least it is accessible to such measures, but if they seem too drastic, by all means pay a visit or attend a conference instead.

Yours faithfully,

J. M. RICHFIELD

Cobham, Surrey, UK

Radioactive whales?

SIR,—It occurred to me recently that the public fear of radioactivity, irrational as it is, might be made to serve a useful purpose. Many of us have been greatly moved by the efforts of organisations such as Greenpeace to save the whales and seals from extinction by brutal commercial interests. Why not make a few radioactive?

A radioactive material might be injected from a distance with the type of hypodermic dart used to tranquillise animals. There are several isotopes such as tritium, sulphur-35, iron-55, carbon-14 and so on which emit radiation sufficiently soft to be shielded even by the syringe wall but which can be presented in a form such that all the tissues of an animal become labelled a short time after injection. The isotope could be changed every year to provide valuable tracer information. It would probably be necessary to label only a dozen or so of each threatened species. The word would get around faster than Arab mercury in Israeli oranges and humanity would not approach those species again in our lifetime.

Yours faithfully,

MALCOLM THACKRAY

Menlo Park,
California, USA

Misleading headlines

SIR,—I read with interest the editorial discussing ambiguous or misleading headlines in newspapers and magazines (20 October, page 637). It seems that the headlines game is being played by nearly everybody but not, as you so carefully point out, by you. Imagine my surprise when I turned to page 644 of this same issue and began to read the article entitled 'Space retrieval' expecting a discussion of, say, satellite recovery or the Space Shuttle's reusable launch rockets, only to find that it was a report on the Soviet attempt to save face after Sovuz 25 failed to dock with Salyut 6. It appears that even the *Nature* reader does not always know what he is getting.

Yours faithfully,

JEFFREY W. PERCIVAL

University of Wisconsin, USA

news and views

In vitro mutations in the initiation codon

from Pamela Hamlyn

THE translation of messenger RNA into protein by ribosomes is usually initiated not at the 5' end of the mRNA, but some way in, resulting in a stretch of nucleotides, the 5' untranslated region, before the coding region of the mRNA. The 5' untranslated region varies in length in different messengers. The ribosome must, therefore, 'select' the part of the mRNA where translation should begin. By binding ribosomes to mRNA, but preventing their movement along the message and removing uncomplexed mRNA with ribonuclease, it has been possible to delineate a region known as the ribosome binding site. It was hoped that characterisation of this region of mRNA might explain its affinity for ribosomes. The ribosome binding site spans the 5' untranslated region and part of the coding region. Roughly in the centre is the initiation codon AUG, the first codon to be translated, directing formyl methionine to the N-terminal position of the protein. Another feature of the ribosome binding site, at least in prokaryotic mRNAs, is a purine-rich region which precedes the initiation codon and shows complementarity to the 3' end of the 16S RNA (the 'Shine and Dalgarno' interaction).

The small bacteriophage Q β is often used as a model for prokaryotic protein synthesis. Its genetic material is single-stranded RNA with three cistrons arranged 5' A α -coat-replicase 3'. A 'read through' of the termination codon for the coat cistron produces a large protein A α . The RNA which infects *E. coli* (the plus strand) acts as an RNA for phage proteins and also as a template for the synthesis of a complementary minus strand. The minus strand is replicated in turn to produce plus strands which combine with the phage proteins to form infectious particles.

Mutations in the initiation codon

cannot be studied by classical procedures, since, with the possible exception of AUG $\rightarrow$ GUG, they may be expected to be unconditionally lethal. C. Weissmann and his colleagues at the University of Zürich, in order to obtain mutations in this region, have exploited Q β 's mode of replication to perform what they call 'site-specific mutations'. Using this technique they have produced a mutation in the AUG initiation codon of the coat cistron and the nucleotide (G) immediately adjacent to it on the 3' side (Taniguchi & Weissmann *J. molec. Biol.* **118**, 533; 1978). Plus strands were isolated and transcribed *in vitro* into minus strands. It is possible to synchronise the synthesis of the minus strands. When the AUGG (the initiator codon of the coat cistron and next nucleotide) are being copied, stepwise synthesis allows the insertion of an analogue N⁴-hydroxycytosine monophosphate opposite the Gs instead of the normal cytosine monophosphate. Synthesis of the minus strand is continued to completion by diluting out the analogue with normal CTP. These minus strands are then used as template for the *in vitro* synthesis of plus strands. N⁴-hydroxycytosine in the minus strand directs the insertion of both A and G into the plus strand, resulting in four possible arrangements at the coat cistron initiation region, namely AUGG (wild type) and AUGA, AUAG and AUAA. The four different types of plus strand were not isolated but their relative proportions were calculated. To determine the ribosome binding capacity of the different mutants, excess ribosomes were bound to the mixed plus strands and ribosome binding sites isolated. Nucleotide sequence analysis was used to calculate the ratio of the different plus strands involved in ribosome binding. Correcting for the original differences in concentration of the mutant plus strands allowed the calculation of values for the relative efficiency of ribosome binding. The results were surprising. As might be expected, the mutants in which the

initiation codon was changed to AUA, that is, AUAG and the double mutant AUAA, were less efficient at ribosome binding than the wild type AUGG. However, of the two the double mutant AUAA showed better binding efficiency than AUAG which in fact does not bind to any detectable extent. On the other hand the mutant AUGA shows at least 50% increase over wild type (AUGG) in ribosome binding efficiency. It seems, therefore, that an A, rather than the G, which is normally found in the fourth position of the coat cistron, enhances ribosome binding to the extent of making a codon AUA, which otherwise cannot participate in ribosome binding, able to do so, and to improve the natural binding capacity of the wild-type initiation codon.

In seeking to explain the beneficial effect of an A rather than a G next to the initiation codon, the authors examined the sequence of nucleotides in the anticodon loop of fMet-tRNA with which the AUG forms base-pairs. They noticed that the replacement of a G by an A allowed a fourth base pair to be formed between the coat cistron initiation region and the tRNA. The authors conclude that the interaction between the ribosome binding site and fMet-tRNA is essential for 70S complex formation, at least in the case of Q β coat cistron.

The ribosome binding site in eukaryotic cells has also been studied. There seems to be considerable sequence variation in the 5' untranslated region, the only recognisable common features being the AUG initiation codon and a 5' terminal methylated 'cap' structure (m⁷GpppX . . .). The significance of the cap structure is difficult to assess. Kozak and Shatkin at the Roche Institute of Molecular Biology have concluded in their latest publication (Kozak & Shatkin *Cell*, **13**, 201; 1978) that its contribution to the formation of initiation complexes is quantitative rather than qualitative. In their recent experiments they have assessed the

capacity for ribosome binding of different size fragments prepared from the 5' end of reovirus mRNA. Fragments up to 30 nucleotides in length, which contained the cap but not the initiation codon, did not form stable complexes with ribosomes whereas every fragment which did contain the AUG was able to bind ribosomes.

However, binding efficiency was reduced by removal of either the cap or of sequences comprising the beginning of the coding region. Their conclusion is that, as with prokaryotic ribosome binding sites, the formation of the initiation complex is primarily dependent on the interaction between the AUG and the initiator tRNA. □

Mechanism of transcription termination

from Andrew Travers

The regulation of transcription termination is a crucial aspect of the control of gene expression in bacteria and bacteriophage. The termination of an RNA chain requires first that the transcribing polymerase ceases its movement along the DNA template, then that both the completed RNA molecule and RNA polymerase be released. *In vitro* the DNA sequences signalling termination differ in their effectiveness. At some sites RNA polymerase can terminate by itself, at others RNA release requires an additional protein factor. However, polymerase can still pause at such ρ -dependent sites even in the absence of the factor.

What is the nature of the termination signals? Analysis of several ρ -independent terminators has revealed that in every case termination occurs distal to a GC-rich region within a run of uridine residues. In addition all such terminators display a region of dyad symmetry in the DNA just proximal to the termination point. In terms of RNA structure this implies that the molecule has the potential to form a stem-loop configuration close to the 3'-terminus. The stability and size of these structures is variable, termination of those RNA molecules possessing the least stable stems being in general more dependent on ρ .

The advent of modern techniques has considerably enhanced the direct sequence analysis of DNA regions with particular regulatory functions. In this issue of *Nature*, (page 414 and 410), Rosenberg *et al.* and Schwarz *et al.* present DNA sequences from phage encompassing the first terminator, t_{RI} , for RNA molecules initiated at a major early promoter, P_R , while Küpper

et al. (page 423) describe the entire sequence of the $tRNA^{Tyr}$ gene distal to the promoter. Termination at both t_{RI} and the major $tRNA^{Tyr}$ terminator is absolutely ρ -dependent. The most striking difference between these terminators and the ρ -independent terminators so far studied is the absence of a terminal run of uridine residues. However t_{RI} does contain a sequence for a comparatively abbreviated stem-loop structure just before the 3'-end of the transcript. The functional significance of this region is made apparent by mutations which alter the effectiveness of the t_{RI} terminators. The *cin* and *cnc* mutations both act in *cis* to enhance or eliminate respectively ρ -dependent termination at this site. The single base change in the *cin* mutant extends the length and stability for the potential stem-loop structure while, by contrast, those of the *cnc* mutants reduce its stability. Accordingly, Rosenberg *et al.* speculate that the stability of this structural feature is an important determinant of termination efficiency although they point out that the *cin* mutation could exert its effect in another way by generating a sequence believed to be important in promoter function and thus stabilising polymerase bound at t_{RI} .

By contrast at the main $tRNA^{Tyr}$ terminator no such stable stem-loop structure is apparent. Instead a cluster of four G-C base pairs precedes the termination point by one turn of the DNA double helix, a configuration believed to be sufficient to halt transcription temporarily (Maizels *Proc. natn Acad. Sci. U.S.A.* **70**, 3585; 1973). In addition a more extensive GC-rich region follows the termination point. Consequently Küpper *et al.* suggest that a transcribing polymerase can be halted either by a stem-loop structure in the product

RNA or by a stable helical region in the DNA template. However, it is clear that such stable regions are by themselves probably insufficient for chain release to occur. Within the $tRNA^{Tyr}$ gene a 178 base pair unit is completely repeated three times. Each of these repeats contains both the proximal and distal GC-rich sequences in their entirety. Nevertheless only two out of the three such regions can apparently serve as terminators *in vitro*. The region that is inactive differs by a C→T transition from the other two, a change that occurs in a sequence CAATCAA common to both the $tRNA^{Tyr}$ and t_{RI} terminators. Küpper *et al.* suggest that this sequence may be the recognition site for ρ -induced chain release.

In phage λ the control of terminator function is believed to have a crucial role in the lytic and lysogenic pathways of development. Thus expression of the genes distal to t_{RI} is dependent on the function of the N gene product, a protein whose postulated role is that of an anti-terminator at a majority of termination sites (Roberts *Nature* **224**, 1168; 1969). pN will however only mediate antitermination when transcription originates from either of the two early promoters suggesting that recognition sites for pN (*nut*) are situated in reasonable proximity to these promoters. One such site *nut_R*, has been identified by Rosenberg *et al.*, as a region of dyad symmetry immediately preceding the terminator t_{RI} . Thus the regulator recognition signal and its site of action are, in this instance, in close juxtaposition.

The sequence analysis of the λ genome provides tantalising hints about the mechanism of the choice between the lytic and lysogenic modes of development. The *cin* and *cnc* mutants favour the lysogenic and lytic modes respectively, suggesting that reduction of transcription beyond t_{RI} allows transcription to proceed in the reverse direction from the promoter P_{RE} whose transcript acts as a messenger for the production of cI repressor. Rosenberg *et al.* and Schwarz *et al.* have also defined the sites of the *cY* mutations, whose phenotypic consequence is the reduction of repressor synthesis during the establishment of lysogeny. These *cis* acting mutations apparently define a regulatory sequence and again occur in regions of dyad symmetry. However it remains unclear whether the *cY* region is the promoter site for P_{RE} , a terminator site for a transcript initiated from P_{RE} , or a regulator recognition site. The identity of one section of the *cY* region with part of the t_{RI} region may indicate a common function but clearly the precise elucidation of the function of these sequences awaits a rigorous biochemical analysis. □

Andrew Travers is at the MRC Laboratory of Molecular Biology, Cambridge.

Does Mars have a magnetosphere?

from Alan Johnstone

THE flow of magnetised plasma outward from the Sun, known as the solar wind, is supersonic with a Mach number of 6 to 8 in relation to planetary bodies it encounters. If it is deflected around a planet it forms a bow shock wave whose shape can be deduced by analogy with the flow of a gas around a suitably-shaped blunt body. Shock waves have been detected up-stream from Mercury, Venus, Earth, Mars and Jupiter but not the Moon because it absorbs all the plasma without deflecting it. For all these bodies, except Mars, the means by which the solar wind is deflected is known in principle if not in detail. Mercury, Earth and Jupiter deflect it magnetically, forming a magnetosphere whose boundary, or magnetopause, is found where the magnetic pressure exerted by the dipolar magnetic field balances the pressure of the solar wind. At Venus, the ionosphere forms a barrier to the flow as its magnetic field is too weak to deflect the solar wind before it encounters the atmosphere.

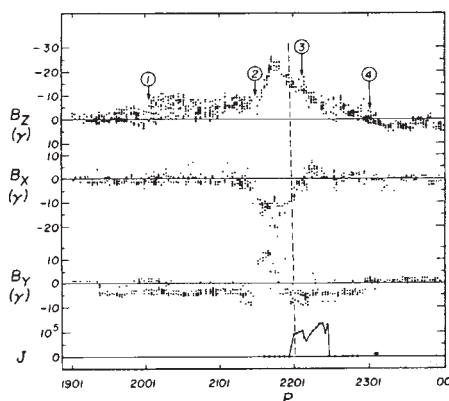
Mars remains controversial because although it has a thin atmosphere it is not known whether its intrinsic magnetic field is sufficiently strong to deflect the solar wind first. The only evidence for a Martian magnetic field comes from measurements made in the interaction region by three Russian spacecraft which orbited the planet. Russell (*Geophys. Res. Lett.* **5**, 81; **5**, 85; 1978) has criticised the interpretation of Dolginov (*Geophys. Res. Lett.* **5**, 89; **5**, 93; 1978) who claims that Mars has a magnetosphere. The Mars 2 and Mars 3 spacecraft crossed the shock wave on the dayside and approached the planet on the sunward side. Mars 5 crossed the shock wave near the terminator and then dipped into the magnetic tail or wake behind the planet. The different sets of data raise different issues and have been treated separately.

The argument over Mars 2 and Mars 3 initially concerns the identification of the shock wave. Knowing the Mach number of the flow and an approximate shape for the obstacle, the size of the obstacle can be deduced from the position of any shock traversal relative to the centre of Mars using the gas-dynamic analogy. If the

obstacle height is found to be more than 400 km or so above the surface of Mars then it is almost certainly magnetospheric. The dipole moment of Mars can then be calculated from the obstacle height and solar wind pressure. Dolginov places the shock wave at the point where the magnetic field first increases as the planet is approached (point 1 in the figure) and claims that between 2 and 3 the spacecraft is within the Martian magnetosphere. The shock front can also be detected in plasma measurements made by Bogdanov and Vaisberg (*J. geophys. Res.* **80**, 487; 1975) and Gringauz *et al.* (*J. geophys. Res.* **81**, 3349; 1976) by a broadening of the ion velocity distribution as the directed flow of the solar wind is thermalised in the shock. The location of the shock obtained this way lies close to the point identified as the magnetopause by Dolginov. The broadening of the ion velocity distribution is a necessary feature of the shock to allow for deflection of the flow and is therefore a more reliable indication. The magnetic field disturbance can be caused by waves propagating upstream from the shock. Russell uses the plasma data of Gringauz *et al.* to obtain a mean obstacle height of 400 km. There is a great deal of scatter in the individual points.

It may be thought that the increase in magnetic field as the satellite

Mars 3 magnetogram of 21 January, 1972. The *X* direction is towards the Sun. The *Z* direction is towards the north pole of the ecliptic and the *Y* direction completes a left-handed coordinate system. Circles 1 and 4 indicate bow shock crossings, 2 and 3—magnetopause crossings. The lower curve shows the ion flux from 0.3–0.15 keV. (from Dolginov *Geophys. Res. Lett. op. cit.*)



approaches Mars is a sure sign of an intrinsic magnetic field but there are two alternative explanations. First, as the solar wind passes through the shock it is compressed and the magnetic field is thereby increased. In pictorial terms, the field lines pile up as they encounter the obstacle, they become draped over its surface and eventually slip away to the side and drift downstream. Second, if there is sufficient conductivity in the Martian ionosphere, currents will be induced in the surface to prevent the penetration of solar wind field into the ionosphere. Russell argues that the configuration of the magnetic field between points 2 and 3 in the figure is what would be expected if the field lines were draped over the obstacle. Dolginov tries to fit these measurements to a dipolar field but the inconsistency of the results raises grave doubts about their significance.

Mars 5 provided more observations of the shock, and dipped into the wake behind the planet. In the wake region the plasma flow velocity is much lower and possibly isotropic and the magnetic field less variable than in the shocked solar wind flow. Dolginov argues that the region is part of the magnetic tail of the planet because the direction of the radial component stays the same even when the magnetic field in the solar wind reverses its direction. Russell points out that if the field in the tail was of Martian origin it should be predominantly radial whereas the measured field is more consistent with the configuration of field lines draped around the obstacle. Russell concludes that none of the probes has detected an intrinsic Martian magnetic field and that an upper limit to its possible value can be placed at 2×10^{21} gauss cm³. Dolginov insists that both sets of data are consistent with a Martian dipole moment of 2.2×10^{22} gauss cm³—an order of magnitude higher than Russell's limit.

The controversy will not be resolved until accurate plasma and magnetic field measurements are made nearer to the Martian surface. It is surprising to find that no plasma or magnetic field measurements have been made closer to the planet than 1,100 km. If the solar wind is deflected by an obstacle at 400 km measurements are needed below this level to identify the nature of the interaction and establish the value of the Martian dipole moment. Even if the planet has a smaller dipole moment than Dolginov estimates, the magnetic field could still have an important role in the interaction and make the Martian ionosphere/magnetosphere system an especially interesting case to study. □

Alan Johnstone is at the Mullard Space Science Laboratory of University College, London.

Seismic stratigraphy and global changes

from A. Hallam

A MAJOR breakthrough in data processing in the 1960s has made seismic reflection profiling an immensely valuable tool in exploration for petroleum. Superbly clear representations of subsurface structures and stratal dispositions are now available from extensive sectors of the continental shelf and slope, as well as under the land surface, and have become virtually indispensable as guides to where the initial exploratory boreholes should be drilled.

For the last decade Peter Vail and co-workers at the Exxon exploration laboratories in Houston have been engaged in an extensive, world-wide programme of stratigraphic analysis of seismic reflection profiles and have come up with some impressive results. Vail has been lecturing frequently in the past few years in North America about the most striking of these, which support the concept of a series of eustatic cycles through the Phanerozoic, and to say that publication has been eagerly awaited is for once an understatement. This has now been achieved with the appearance last December of Memoir No. 26 of the American Association of Petroleum Geologists.

It was the eminent Austrian geologist Eduard Suess who at the turn of the century coined the term 'eustatic' for world-wide changes of sea level. While there has been universal acceptance of this phenomenon for the Pleistocene, with sea level falling and rising as a consequence of the waxing and waning of polar ice caps, the postulation of global sea level changes in earlier times has proved more controversial. It has been difficult, for instance, to persuade the sceptics that a given marine transgression in the region they were well acquainted with was a local expression of a eustatic rise rather than the consequence of a local increase in tectonic subsidence rate. The critical requirement is of course good biostratigraphic correlation, and as this has improved over the years there has been a gradual shift of opinion in favour of eustasy.

The importance of the new work in seismic stratigraphy is that a vast amount of new data has become available for analysis. What is particularly significant is that seismic reflections tend to parallel stratifications rather than gross boundaries of lithologic units that may cut across

stratification surfaces; hence they have chronostratigraphic value. Age and facies control must of course be achieved independently by study of fossils and sediments from boreholes.

The essence of the technique adopted by Vail and his colleagues is to define so-called 'depositional sequences', which are major unconformity-bounded stratal units, in a given region. Relative rise of sea level is indicated by coastal onlap of maritime deposits, stillstand by coastal topset and relative fall by downward shift of coastal onlap. 'Sea level curves' are constructed by making chronostratigraphic correlation charts of successive depositional sequences and plotting the apparent rise and fall of sea level through time (correction is made as far as possible for differential subsidence). If good correlation exists between three or more regions in different parts of the world, eustatic control is inferred.

The end product of the exercise is a chart of global cycles, which is a modal average of regional cycles. Although the whole Phanerozoic is included, pre-Jurassic data are based entirely on North America and hence cannot be considered as reliable as those from the Jurassic to the present, which come from six continents. These show an overall rise of sea level from the early Jurassic to the late Cretaceous, followed by an overall lowering through the Cainozoic, made up of a series of eustatic cycles characterised by a gradual rise, period of stillstand and rapid drop. Calibration with a quantitative model of W. C. Pitman relating oceanic ridge volume to sea floor spreading rates suggests a drop of about 350 m from the late Cretaceous maximum some 80 Myr ago. A particularly dramatic drop of a few hundred metres early in the late Oligocene (30 Myr) is thought to have been accomplished within the limits of biostratigraphic resolution, about a million years.

There can be little dispute that this work represents one of the most significant advances in stratigraphy for many years, but problems quite

naturally remain. For instance, if we are to accept the reality of geologically frequent rapid drops of sea level, what can the causal mechanism be? It is not plausible to invoke glaciation before the late Cainozoic because there is no evidence of polar ice caps, but on the other hand changes of oceanic ridge volume appear to happen too slowly to account for the dramatic phenomenon in question.

A more serious difficulty perhaps concerns the reproducibility of the results. The raw data on which the analysis is based are likely to remain unpublished, having been classified as confidential. Would research groups from other oil companies come up with a closely similar chart? What, finally, of the poor old academic geologist who is denied access to the critical data—how much should he take on trust? We must hope that information will be released into the public domain in the tolerably near future because it is scientifically too valuable to be allowed to gather dust in unopened files. □

When C₄ plants do best

from Peter D. Moore

As more information accrues on the metabolic implications of the C₄ photosynthetic pathway, ecologists and biogeographers are presented with a variety of interesting problems. In how many taxonomic and geographic localities has this technique for accumulating CO₂ evolved? Under what environmental conditions does the C₄ system confer maximum advantage? Why are C₄ species so scarce in temperate latitudes? If the C₄ plants are as efficient as we are told in tropical and subtropical, dry environments, why have they not totally eliminated their C₃ counterparts? Some of these questions have recently been raised and possible answers constructed by Ehleringer (*Oecologia (Berl.)* 31, 255; 1978).

In particular, Ehleringer is concerned with the reduced quantum yield for CO₂ uptake in C₄ plants which he and Björkman have examined (*Plant Physiol.* 59, 86; 1977). The C₄ system, which involves the temporary fixation of CO₂ into a four carbon molecule, its transportation to the vascular bundle sheath cells and its subsequent release and refixation by the conven-

A hundred years ago

THE SATELLITES OF MARS—PROFESSOR ASAPH HALL, to whom as the discoverer of these bodies, the right of selection of names appertains has definitely decided for *Deimos* for the outer moon and *Phobos* for the inner one, agreeably as he mentions to the suggestion of Mr Madan in these columns, founded on the lines in the "Iliad", which Pope thus renders:—

"With that he gives command to Fear and Flight
To join his rapid coursers for the fight;
Then grim in arms, with hasty vengeance flies,
Arms that reflect a radiance through the skies."

From *Nature* 17, 28 March, 433; 1878.

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tional Calvin cycle pathway, involves additional energy expenditure over the simpler C_3 system. Investigations of the quantum yield (CO_2 fixed for a given light absorption) of C_3 and C_4 plants have produced contradictory findings, but the results of Ehleringer and Björkman on a variety of C_3 and C_4 species using intact, attached leaves seem to have clarified the issue. In an atmosphere of 21% oxygen a C_3 plant, *Encelia californica*, showed a quantum yield which was markedly dependent on temperature. Oxygen inhibition (photorespiration) increased with temperature (between 14 and 38 °C), hence quantum yield decreased with temperature over this range. A C_4 species, *Atriplex rosea*, showed no such dependence of quantum yield upon temperature. Between 12 and 37 °C in a 21% oxygen atmosphere, its quantum yield remained constant. At lower temperatures (below about 28 °C), however, the quantum yield of the C_4 plant was lower than that of the C_3 plant, the reverse being true at higher temperatures. The lack of temperature dependence of quantum yield in the C_4 plant results from the absence of photorespiration, and its lower quantum yield at low temperatures is probably due to the higher energy demand of this technique of carbon accumulation.

Thus at about 28 °C the additional ATP requirement of the C_4 plant is almost precisely offset by the oxygen inhibition experienced by C_3 plants, thus giving them identical quantum yields. At higher temperatures the C_4 plant has the edge in terms of energetic efficiency, but at lower temperatures the C_3 plant is more efficient, assuming that other conditions, such as soil moisture and atmospheric CO_2 levels, are adequate. At low oxygen levels the C_3 plant gains an advantage, since photorespiration declines, while at low CO_2 concentration the C_4 plant is favoured since it possesses the facility for carbon concentration in its vascular bundle sheaths.

The critical importance of leaf temperature in determining the relative efficiency of the two systems has important ecological and biogeographical implications. One would expect C_4 plants to be at an advantage in high illumination, high temperature environments and C_3 plants to be more efficient at lower temperatures and under dense canopies. Observations generally bear out such a prediction, though there are exceptions, such as the C_4 species of *Euphorbia* described by Pearcy and Troughton (*Plant Physiol.* **55**, 1054; 1975) from Hawaiian rain forests. Some of these *Euphorbia* species are small trees and shrubs growing under the shade of forest canopies.

Where C_3 and C_4 species occur

together, one may find a niche separation in temporal terms, as in some prairie grasses where C_3 species are more active in their growth during cooler periods and C_4 species attain greater importance when conditions are hot and dry (Williams *Plant Physiol.* **54**, 709; 1974).

It is this discovery that C_4 plants are not superior under all circumstances which has led Ehleringer to construct mathematical models of grass canopies, so that the total plant carbon economy, including well lit and shaded leaves, can be studied under a variety of environmental conditions. Grass canopies were chosen because this life form is common to both C_3 and C_4 species, they range over a wide variety of climatic regimes and their high leaf area index would make them particularly sensitive to variation in quantum yield. The two models constructed, for C_3 and C_4 canopies, differed simply in their dependence of quantum yield and light-saturated photosynthesis on temperature.

Several types of environmental simulation were attempted. A transect of the Great Plains area was simulated in terms of July temperatures. It was found that C_4 canopies had a theoretical advantage at latitudes below 45 °N and C_3 canopies were superior with respect to rate of carbon gain at higher latitudes. The Sonoran desert experiences two periods of rainfall, November to March and July to September. Ehleringer's simulation study suggests that the C_3 canopy is at an advantage during the November to March period, but that for the rest of the year the C_4 canopy is superior. Finally he has examined a shaded habitat, receiving only 10% of available light. Under these conditions the C_3 canopy exceeded the C_4 canopy in its carbon gain up to a temperature of about 33 °C. Above that temperature, the C_4 canopy is theoretically superior despite the low light, up to 44 °C when net gain falls to zero.

These simulation predictions conform closely with field observations. The proportion of C_4 grasses in the canopy of Great Plains grassland is higher (>50%) in sites around 40 °N than at latitudes above 45 °N (<50%). In the Sonoran Desert, the winter grasses are C_3 and the summer ones C_4 . The shade studies also explain the lack of C_4 species as understorey components of stratified habitats, with the exception of the Hawaiian *Euphorbia* species which can be accounted for by the high temperatures experienced.

Thus the present biogeographical distribution pattern of C_4 photosynthesis does seem to reflect the selective advantage of the system given certain environmental conditions. High light,

high temperature and water shortage provide a strong selective advantage to the species possessed of this attribute. Under cooler or shaded conditions, however, the high energy costs of the C_4 system evidently prove to be a positive handicap to a species in competition with a conventional C_3 plant. □

Core compression in nuclei

from P. E. Hodgson

There has been for many years a troublesome discrepancy between the Coulomb energy differences of neighbouring nuclei and the root mean square radii of the neutrons and protons outside the core. If the Coulomb energies are calculated from the known radii they are about 7% smaller than the measured ones, whereas the measured Coulomb energies correspond to excess neutron and proton radii that are similar to the total proton radii, contrary to shell model calculations. This discrepancy is known as the Nolan-Schiffer anomaly.

There is also a problem about the magnitude of the difference between the root mean square radii of all the neutrons and of all the protons in nuclei. This quantity is not easy to measure accurately, but recently a number of quite different methods have given results that are substantially less than the values given by the Hartree-Fock theory.

In a recent paper, Shlomo and Friedman (*Phys. Rev. Lett.* **39**, 1180; 1977) have shown that these anomalies are connected, and that both are simultaneously resolved if account is taken of the compression of the nuclear core by the neutrons outside the core. In the usual formula for the Coulomb energy difference it is assumed that the root mean square radii r_p and r_n of the protons and neutrons in the core are the same, and that the corresponding radii r_x of the protons and neutrons outside the core are also the same. Evidence has recently been mounting that neither of these assumptions is sufficiently accurate and by relaxing them Shlomo and Friedman were able to obtain a consistent account of Coulomb energies and nuclear radii.

As an illustration of the existing anomalies, they discuss several nuclei in detail. The experimental values of $\Delta r = r_n - r_p$ for ^{48}Ca and ^{208}Pb are about 0.1 and 0 fm respectively, whereas the Hartree-Fock and simple shell model calculations give 0.2 to 0.3 fm. For several other isotopes, the

Hartree-Fock values of r_p are about 30% less than the experimental values whereas the values of r_{ex} are somewhat larger. In order to obtain the experimental values of the Coulomb energy shift, the values of r_p have to be close to the corresponding values of r_{ex} , in disagreement with both theory and experiment. Thus to give the experimental value $E_c = 7.28$ MeV for the ^{41}Sc - ^{41}Ca ground states r_{ex} must be as small as r_p , that is about 3.5 fm. There are many higher order effects, but these discrepancies persist even when they are taken into account.

These difficulties vanish when allowance is made for core compression. Talmi and Shlomo have shown that the change in the Coulomb energy difference due to the compression of the proton core is

$$\Delta E_{cc} = (3/5)^{3/2} e^2 (Z(Z-1))/r_p \cdot (\Delta r_{cc})/r_p$$

where $\Delta r_{cc} = r_p - r_p'$ is the change in radius of the core. Applying this to the case of ^{41}Sc - ^{41}Ca and taking

$r_{ex} = 4.2$ fm for the $1f_{7/2}$ valence proton in ^{41}Sc shows that a compression of $\Delta r_{cc} = 0.04$ fm corresponds to a change in the Coulomb energy difference of about 0.8 MeV, which is sufficient to account for the discrepancy.

The above formula for the effect of the core compression assumes that the proton and neutron radii of the core are equal; this is usually very nearly the case, but calculations can be made to take account of finite values of Δr . These show that the core compression effect increases with decreasing r and with increasing r_{ex} . Thus in the case of ^{40}Ca and ^{48}Ca , whose proton radii are equal to within 0.1 fm it is found that the 20 neutrons in the core of ^{48}Ca are compressed relative to those in the core of ^{40}Ca by more than 0.1 fm. The corresponding proton core compression is sufficient to resolve the anomaly in the Coulomb energy. $\square$

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Chemoprevention of cancer

from Michael B. Sporn

THE importance of cancer prevention, as distinguished from cancer treatment, has become increasingly apparent in recent years. A workshop held recently at the National Institutes of Health* discussed pharmacological and nutritional approaches to the prevention of cancer. All papers dealt with prevention of disease, and the treatment of invasive malignancy was not considered. Although prevention of exposure of people to carcinogens is theoretically the best way to reduce cancer incidence, such a strategy is not always feasible. Therefore, alternative ways to modify the process of carcinogenesis in face of exposure to carcinogens or promoters must be found as a practical means to prevent the development of invasive cancer in the human population. Since carcinogenesis is a prolonged, multistage process, a variety of approaches may be considered towards either the inhibition of the initiation phase or the inhibition of the promotion (progression) phase of carcinogenesis.

Inhibition of initiation of carcinogenesis may be directed toward: the inhibition of formation of carcinogens from dietary constituents, the inhibition of metabolic activation of carcinogens, or the inhibition of binding of activated metabolites to critical cellular targets, such as DNA. S. Mirvish (University

of Nebraska) summarised extensive studies which show that ascorbic acid blocks nitrosamine formation from secondary amines and nitrite, by causing breakdown of nitrite. Since nitrite can be formed from bacterial reduction of nitrate (found in many foods) in the mouth, the problem of nitrosation of secondary amines to form potentially carcinogenic nitrosamines in the gastrointestinal tract, would not totally disappear with the elimination of nitrite as a food preservative. J. Weisburger (American Health Foundation) stressed the importance of a low fat, high fibre diet in prevention of colon carcinogenesis. A high fat intake causes greater faecal excretion of several bile acids, which have been shown to be promoting agents for colon carcinogenesis in the rat. High fibre increases the total bulk of faecal contents, which results in dilution of carcinogenic substances that might be present in the colon. W. R. Bruce (Ontario Cancer Institute) reported on the presence of mutagenic substances (assayed by the Ames test) in human faeces. The chemical nature of these materials has yet to be determined, as has their possible causative role in human colon cancer. Feeding of high amounts of ascorbic acid was found to lower the level of faecal mutagen found in several normal human subjects.

The use of various phenolic antioxidants, including the food additives BHA and BHT, to inhibit carcinogen activation and binding to cellular targets was discussed by L. Wattenberg and L. Lam (University of Minnesota) and T. Slaga (Oak Ridge National Laboratory). BHA and BHT both cause significant inhibition of binding of polycyclic hydrocarbons to DNA in several test systems; and E. Weisburger (National Cancer Institute) and H. Black (Baylor University) reported that BHT can inhibit liver carcinogenesis (initiated by aromatic amines) and skin carcinogenesis (initiated by ultraviolet light), respectively. The role of selenium and its salts as inhibitors of carcinogenesis was discussed by A. C. Griffin (M.D. Anderson Hospital) and G. Schrauzer (University of California, San Diego). Epidemiological data indicate an inverse relationship between environmental selenium and rates of colo-rectal and breast cancer in men and women, and several experimental studies indicate that addition of selenium salts (1-4 p.p.m.) to the drinking water of rats or mice reduced incidence of either spontaneous or chemically-induced tumours of the breast, liver, and colon. The mechanism of the protective effect of selenium is not understood, and it was emphasised that high doses of selenium can be extremely toxic.

The most useful agents for inhibition of promotion of carcinogenesis have been the retinoids (analogues of vitamin A), which have been used to prevent cancer of the skin, lung, bladder, and breast in experimental animals. M. Sporn (National Cancer Institute) stressed the hormone-like action of retinoids in controlling cell differentiation, and the practical need for synthesis of new retinoids which will be more potent and have less toxicity when given chronically. The requirement for chronic administration of retinoids to suppress chemically-induced mammary carcinogenesis in the rat was reported by R. Moon (IIT Research Institute); his data indicate that the continued presence of high levels of retinoid is necessary for prevention of the phenotypic expression of a genotypic change caused by a carcinogen. Mechanistic studies by R. Boutwell and G. Mueller (University of Wisconsin) show that retinoids are potent anti-promoting agents, particularly in their ability to antagonise the effects of phorbol esters, which are among the most active known tumour-promoting substances.

Clinical application of chemoprevention was considered in the final session of the workshop. J. DeCosse (Medical College of Wisconsin) emphasised the need for safety and freedom from

*February 2-3.

toxicity in any chemopreventive agent, since it would be given to people for long periods of time, several years or even a lifetime in the case of subjects with a high genetic susceptibility to cancer. DeCosse's early studies on patients with multiple polyps of the colon suggest that ascorbic acid can diminish the number of lesions; a definitive randomised double-blind trial on patients in England with familial polyposis of the colon (who develop colon cancer at an early age) is planned in the near future. The mechanism of ascorbic acid in diminishing the number of colonic lesions is unknown, but it may be a result of diminished formation of nitrosamines or some other mutagen, as has been described by Mirvish and Bruce. Another proposed area for clinical studies in chemoprevention was described by W. Koontz (Medical College of Virginia); the National Bladder Cancer Project, Collaborative Group A is about to begin Phase I/Phase II studies of the use of 13-*cis*-retinoic acid to prevent recurrence of bladder lesions in patients who have had surgical removal of early bladder cancers. It was emphasised that there was no indication for the use of the retinoid in treatment of invasive bladder cancer; animal studies have only shown an ability to diminish bladder carcinogenesis in its early, preneoplastic stages. □

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Atmospheres of Uranus and Neptune

from Garry E. Hunt

THE outer Solar System planets Uranus and Neptune resemble each other in many respects and show consistent differences in composition from the other outer Solar System planets such as Jupiter and Saturn. But their resemblance does not apparently extend to their atmospheres as recent results are beginning to show.

Uranus and Neptune are smaller and colder than their giant companions (see for example Hunt *Adv. Phys.* **25**, 455; 1976). Their spectra are dominated by hydrogen and strong methane absorptions in the red and near infrared. Although there are considerable uncertainties in interpreting spectra, pre-

sent analyses suggest that the CH_4/H_2 ratio may be larger than the solar value by as much as an order of magnitude (Cess & Owen *Astrophys. J.* **197**, 37; 1975). It is clear that Uranus and Neptune must have less than solar proportions of the light abundant elements hydrogen and helium, in contrast to Jupiter and Saturn, which apparently are thought to be close to solar composition.

Evidence of other constituents in the planetary atmospheres is extremely limited. There is no information yet on the possible presence of helium, and ammonia has not yet been detected spectroscopically at visible wavelengths (Encrenaz *et al. Astr. Astrophys.* **37**, 49; 1974). Measurements in the microwave portion of the spectrum (Gulkis *et al., Icarus*, in the press) indicate that there may be two orders of magnitude less NH_3 than expected (on the basis of a mixing ratio based on assumed solar abundances in the Uranian atmosphere). Is NH_3 depletion a result of the formation of NH_4SH clouds below the accessible levels of the atmosphere? For this hypothesis to be correct the atmosphere should have a ratio of S to N which is enhanced relative to the solar abundance value. However, sulphur in the atmosphere is most likely to originate through H_2S whose detection is still to be confirmed.

We have no knowledge of ammonia in the atmosphere of Neptune. And recent observations suggest that the assumption that the Neptunian atmosphere would broadly resemble that of Uranus may not be valid. For although Macy and Sinton (*Astrophys. J.* **218**, L79-81; 1977) have detected ethane on Neptune, their simultaneous observations of the Uranian atmosphere failed to detect ethane. Does this suggest differences in the structures of these planetary atmospheres? At first sight this would seem particularly remarkable since the basic properties of these planets are very similar (see Hunt *op. cit.*).

The observations of Neptune by Gillett and Rieke (*Astrophys. J.* **218**, L141-144; 1977), in the ν_1 CH_4 band centred at $7.7\ \mu\text{m}$ indicate a brightness temperature of about 115 K which is much higher than the effective temperature of 55 ± 2.3 K (Loewenstein *et al. Astrophys. J.* **218**, L145-146; 1977). This radiation must be coming from a high level in the atmosphere (this interpretation is consistent with the analysis of the occultation of BD-17° 4388 by Neptune (Veverka *et al. Astrophys. J.* **189**, 569; 1974)) indicating a substantial inversion in the stratosphere. But what is the mechanism responsible for the additional atmospheric heating? In the Earth's atmosphere it is created by solar heating producing an ozone layer. In the atmo-

sphere of Jupiter, Saturn and Titan, solar radiation is absorbed by methane (see for example, Wallace *et al. Astrophys. J.* **193**, 481; 1974; Hunt *op. cit.*; Danielson *et al., Icarus* **20**, 437; 1973). Although methane is plentiful in the atmosphere of Neptune, Wallace (*Icarus*, **26**, 538; 1975) suggests that sufficient additional heating can be obtained only if the atmosphere is supersaturated with it. This would seem unlikely because of the obvious difficulty of maintaining the atmosphere in this enriched state.

The recent far infrared observations of Neptune by Loewenstein *et al. (op. cit.)*, indicate an emission temperature of 55.5 ± 2.3 K compared with a black-body value of ~ 43 – 46 K for a planet at this distance. This clearly indicates that Neptune has an internal heat source. Could this reduce the requirement for methane supersaturation? It seems unlikely, since this additional heating would affect all atmospheric levels rather than one isolated level.

There is, however, a major difference between the Neptunian and Uranian planetary systems, since near Neptune there is a large satellite, Triton, in retrograde orbit, in marked contrast to the Uranian system. Could there be a tidal interaction in which Triton heats the upper atmosphere of Neptune? This is also unlikely, since the most effective heating by this method would take place at very high levels in the atmosphere and at altitudes much greater than the stratospheric region being considered. Consequently, we are left in the difficult position of having found a strong stratospheric inversion in the atmosphere of Neptune, which is inadequately explained by solar heating created by methane absorption and the photochemical aerosols likely to be present at these levels.

But Uranus poses a more complicated problem, for although there is an inversion at stratospheric levels, it is much weaker than that detected in the atmosphere of Neptune. Loewenstein *et al. (Icarus* **31**, 315; 1977) have observed a surface brightness of about 58 ± 2 K, which is approximately the value expected from a radiating black-body at this distance. Uranus may not therefore have an internal heat source, which makes it the only major planet without one. Although its temperature is very similar to the radiating temperature of Neptune, Gillett and Rieke (*op. cit.*) have shown the surface brightness of Uranus to be at least an order of magnitude fainter at $8\ \mu\text{m}$ and about 50 times fainter at $12\ \mu\text{m}$ compared with its planetary neighbour. This suggests an efficient cold trap in the atmosphere, since the methane-to-hydrogen mixing ratio must be much lower on Uranus to account for the weaker inversion. Further anomalies in

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the atmospheric structure of Uranus would result from its large polar inclination of $\sim 98^\circ$, since each hemisphere will spend a substantial period without sunlight during each Uranian year. The north pole of Uranus is just turning towards us, having been turned away from the Sun for more than 40 years. The atmosphere will have cooled substantially in the absence of meridional heat transport. Perhaps at this time the cold hemisphere of Uranus is efficiently absorbing the available solar radiation and therefore radiating less energy than it receives from the Sun.

Certainly, therefore, there are apparently major differences in the structures of the upper atmospheres of these planets. The variations in their individual stratospheric temperature gradients will influence the detectability of minor constituents and account for the observations of Macy and Sinton (*op. cit.*). Furthermore, the absence of a Uranian internal heat source will require any motions in the planetary atmosphere to result from differential solar heating. However, at this distance, the invariant solar radiation is comparatively weak, and we may not anticipate any rapid, large scale, dynamical variations in the atmosphere of Uranus. Neptune, with an internal heat source, could possess some large scale motions. This may then account for the recent perplexing observations of Joyce *et al.* (*Astrophys. J.* **214**, 657; 1977), which indicated a factor of four change in the reflectance of Neptune between 1 and $4\ \mu\text{m}$ during 1975–76, while the simultaneous measurements of Uranus showed negligible change (Hunt *Nature* **269**, 10; 1977). □

Mystery of foetal growth

by Mary Lindley

DURING the past twenty years it has become accepted that some babies are born small not because they are premature but because their growth has been retarded in the uterus. These so-called small-for-dates babies, which are smaller than would be expected for their gestational age, are at greater than normal risk at birth, particularly from asphyxia and hypoglycaemia. They can be treated only by giving them special attention after birth. Earlier intervention is precluded because the mechanisms responsible for intrauterine growth retardation are

poorly understood and it is not even possible to identify a foetus that is growing too slowly. A brave attempt to shed new light on this situation at a recent Dahlem Workshop* seemed to show that the clinical problem remains difficult to approach.

Attempts to define the problem and its causes immediately face the difficulty that although small-for-dates babies generally weigh less than 2.5 kg, they are not easy to identify within the natural variation of a population. As M. Ounsted (John Radcliffe Hospital, Oxford) pointed out, some small babies are perfectly healthy. Her studies of the birthweights of mothers, their infants and relatives have indicated that a woman exerts a characteristic constraint on the foetal growth of her offspring. This property of maternal constraint is passed on to her daughters. The same phenomenon is known in other mammals.

Other factors that can influence total growth are both genetic and environmental. The former include the absence of an X chromosome in females with Turner's syndrome and the presence of a third copy of certain chromosomes, notably numbers 13 and 18. Environmental factors that restrict foetal growth include metabolic maternal diseases and possibly infections. There was some feeling that more attention should be paid to the effects of parasites such as those of malaria, schistosomiasis and trypanosomiasis.

Ionising radiation has restricted foetal growth among Japanese survivors of the atomic bombs. Thus it belongs in the list that includes smoking, alcohol, drugs and other chemicals in food and in the atmosphere, all of which may restrict foetal growth. Participants felt that effects on birthweight may prove to be a sensitive measure of drug toxicity and should perhaps be included in the evaluation of new drugs.

In spite of years of work on the effects of maternal nutrition on the foetus there seems to be no clear picture of this relationship during human pregnancy. Two well-quoted studies suggest that maternal nutrition can limit foetal growth. In the Netherlands during the famine of 1943–44 the birthweight declined 9%, and in a poor rural community in Guatemala supplementary feeding during pregnancy enhanced the birthweight. But such studies are difficult to interpret because of complicating factors, such as disease, which also effect foetal growth. The group at the workshop added no new wisdom and doubtless arguments will continue to

rage. The role of maternal toxemia and hypertension during pregnancy was also disputed, with agreement that severe forms are associated with retarded foetal growth, but less confidence that any effect is evident with milder forms of the disorders.

How and when all these factors exert their effects on foetal growth remains largely a mystery, although a few weak pointers emerged from the discussion. There was much modesty about the dangers of extrapolating from mice, sheep or monkeys to man, with G. S. Dawes (University of Oxford) anxious to outlaw all reference to animal models in this context. Most participants preferred to retain the familiar terminology. One of those was A. Gropp (Medizinischen Hochschule Lübeck) who described his own mouse model. From crosses between *Mus poschiavinus*, the tobacco mouse which has 26 chromosomes, and *Mus musculus*, the house mouse which has 40 chromosomes, he obtains a range of embryos with a single chromosome missing (monosomics) or with an extra chromosome (trisomics). He has found that monosomic embryos show a deficiency of cells before implantation, while after implantation both they and the trisomic embryos are gradually eliminated.

This hint that the effects of chromosome abnormalities on growth can be manifest very early in gestation was one of the few positive answers to emerge from a discussion of whether early development is involved in abnormal foetal growth. For example, an attempt by V. Sara (Karolinska Hospital, Stockholm) to link the current crop of polypeptide growth factors to foetal growth was unsuccessful. She pointed out that as there is no clear evidence that growth factors are involved in post-natal growth it is unlikely that anything could be said about a role for them in the foetus. Similarly no clues about what happens in the growing foetus could be gleaned from the store of knowledge of cell recognition and adhesion.

Prompted by the embryological contributions of R. L. Gardner (University of Oxford) and H. M. Beier (University of Aachen), aficionados of the mouse and rabbit respectively, participants decided that synchronisation between embryo and mother at implantation should be a focus for further studies. Embryo transfer experiments have shown that asynchrony can influence embryonic growth, but it remains to be seen whether the effects continue to be manifest in the growing foetus or whether they simply result in early death. □

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*The workshop was held on 20–24 February in Berlin; proceedings will be published by Dahlem Konferenzen.

articles

Composition waves in iron-doped rutile and the relationship between Young's modulus minima and crystallographic shear orientations

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Three types of long-wavelength sinusoidally modulated crystallographic shear boundaries in reduced rutiles are described. These transverse composition waves represent oscillations within an elastic strain energy potential well produced by the pronounced Young's modulus minimum along $k(010)$.

NEW types of crystallographic shear (CS) behaviour have recently been discovered¹ in iron-doped rutiles. For $\text{TiO}_2 + 14\text{--}16 \text{ wt}\% \text{Fe}_2\text{O}_3$, quenched from $\leq 1,500^\circ\text{C}$, the CS operation is $(020)[0 - 1/2 \ 1/2]$. (All crystallographic indices in this paper refer to the rutile cell or sub-cells.) This composition range is spanned by a series of intergrowths of $9x$ and $11x d_{020}$ CS spacings. Diffuse scattering indicating metal atom ordering within the CS boundaries has now been analysed and complete structural models for the high-temperature structures derived². For $\text{TiO}_2 + 8\text{--}13 \text{ wt}\% \text{Fe}_2\text{O}_3$ a new region of 'swinging shear' behaviour with orientation indices

$$(0kl) = p(020) + q(011) \quad (1)$$

was observed, where p and q are integers with $0.5 \leq p/q \leq \infty$. The CS boundaries rotate from (021) through (031) , and so on to (020) , pivoting about the $[100]$ zone axis. High-temperature $(0kl)$ CS structures transform reversibly into the well-known (hkl) CS structures below approximately $1,450^\circ\text{C}$, with

$$(hkl) = p(121) + q(011) \quad (2)$$

and pivot $[1\bar{1}1]$. An exhaustive X-ray and electron diffraction-high-resolution imaging study of the low- to high-temperature phase transformation mechanism has been made³. We present here observations of new, unsuspected CS phenomena whereby wavelike, sometimes perfectly sinusoidal, variations in CS orientation indices occur. Three distinct types of long-range modulated structures appear during the transformation, with wave vectors $k(101)$, $k(100)$ and $k(001)$. We first give electron diffraction and high-resolution image evidence for the existence and nature of the modulations and then briefly discuss the structures and reaction paths. Examples are drawn from three systems, $(\text{Ti}^{4+}, \text{M}^{3+})\text{O}_x$, with $1.90 < x < 2.0$, $\text{M} = \text{Ti, Fe or Cr}$. Finally we describe a new theoretical approach to the understanding and prediction of CS orientations based on the observation that variations occur about planes normal to directions having minima in Young's modulus, in each of the $[1\bar{1}1][101][001]$ and $[100]$ zone axes. The equilibrium transformation path follows the path of steepest descent from the elastic minimum in the $[1\bar{1}1]$ zone at low temperatures to that in the $[100]$ zone at high temperatures.

Electron diffraction and imaging

Experimental techniques for preparing and thinning the oxides and for high-resolution imaging are described elsewhere¹⁻³ and details of the specimens used here are given in the figure captions. Thin crystals were orientated for symmetrical excitation of $[001]$, $[100]$, $[010]$, $[10\bar{1}]$ and $[1\bar{1}1]$ zones, as required to identify the many different CS orientations and modulations involved.

Low temperature structures: unambiguous identification of (hkl) in equation (2) requires one of the four equivalent $\langle 1\bar{1}1 \rangle$ zone axes. At $\sim 1,300^\circ\text{C}$ all specimens show long rows of superlattice spots parallel to $k(hkl)$ with intensity maxima centred on rutile sub-cell positions. The geometry and indexing of these patterns and phase analyses of the Cr- and Fe-doped systems are given elsewhere⁴⁻⁶.

$k(101)$ modulated structures: Fig. 1a shows a $[10\bar{1}]$ zone diffraction pattern containing $k(101)$ modulation streaking. This is associated with closely-spaced interfaces between CS domains whose indices zig-zag from (121) to $(\bar{1}2\bar{1})$. Remnants of $k(121)/k(\bar{1}2\bar{1})$ superlattice rows are also seen. Eventually both these superlattice spots and the $k(101)$ modulation disappear, leaving only $k(020)$ superlattice rows. Figure 1b shows a high resolution image from a crystal whose $[10\bar{1}]$ diffraction pattern indicated this later stage. Note the oscillatory nature of the CS boundaries relative to mean orientation (020) . This oscillation may be described as a transverse wave propagating along $k(101)$ with amplitude approximately $4.6\text{\AA}$ and wavelength $450\text{\AA}$. In fact, the oscillation is only quasi-continuous because structural units approximately $11.5\text{\AA}$ apart are resolved along the length of the CS boundaries. Resolution of the $4.6\text{\AA}$ d_{010} lattice fringes allows the width of the boundary and the magnitude of the amplitude of oscillation to be determined precisely. The expression 'CS plane' now seems misleading and we have not used it here. The interface between adjacent rutile slabs is complex with projected width ranging from 4.6 to $9.2\text{\AA}$. Since the contrast reversed with change of sign of objective lens defocus, the projected-charge-density image approximation should be appropriate to interpret $5\text{\AA}$ detail⁷. The darker blobs in Fig. 1b, therefore, represent increased metal occupancy of $[\text{MO}_6]$ octahedra in the CS interface. This agrees with the structure determination² which showed that in (020) structures $[10\bar{1}]$ columns of corundum-like structure intergrow with rutile within the CS boundary, whereas the oxygen net is essentially continuous.

$k(100)$ modulated structures: Fig. 2a shows a $[001]$ zone diffraction pattern. Note the modulation sidebands along $k(100)$ and also the $k(020)$ superlattice rows. The $k(100)$ modulation is associated with closely-spaced (100) interfaces between $k(121)$ and $k(\bar{1}2\bar{1})$ domains of low-temperature structure. A range of behaviour is observed where the projection of the superlattice rows in the $[001]$ zone range from $k(120/\bar{1}20)$ to $k(020)$. The

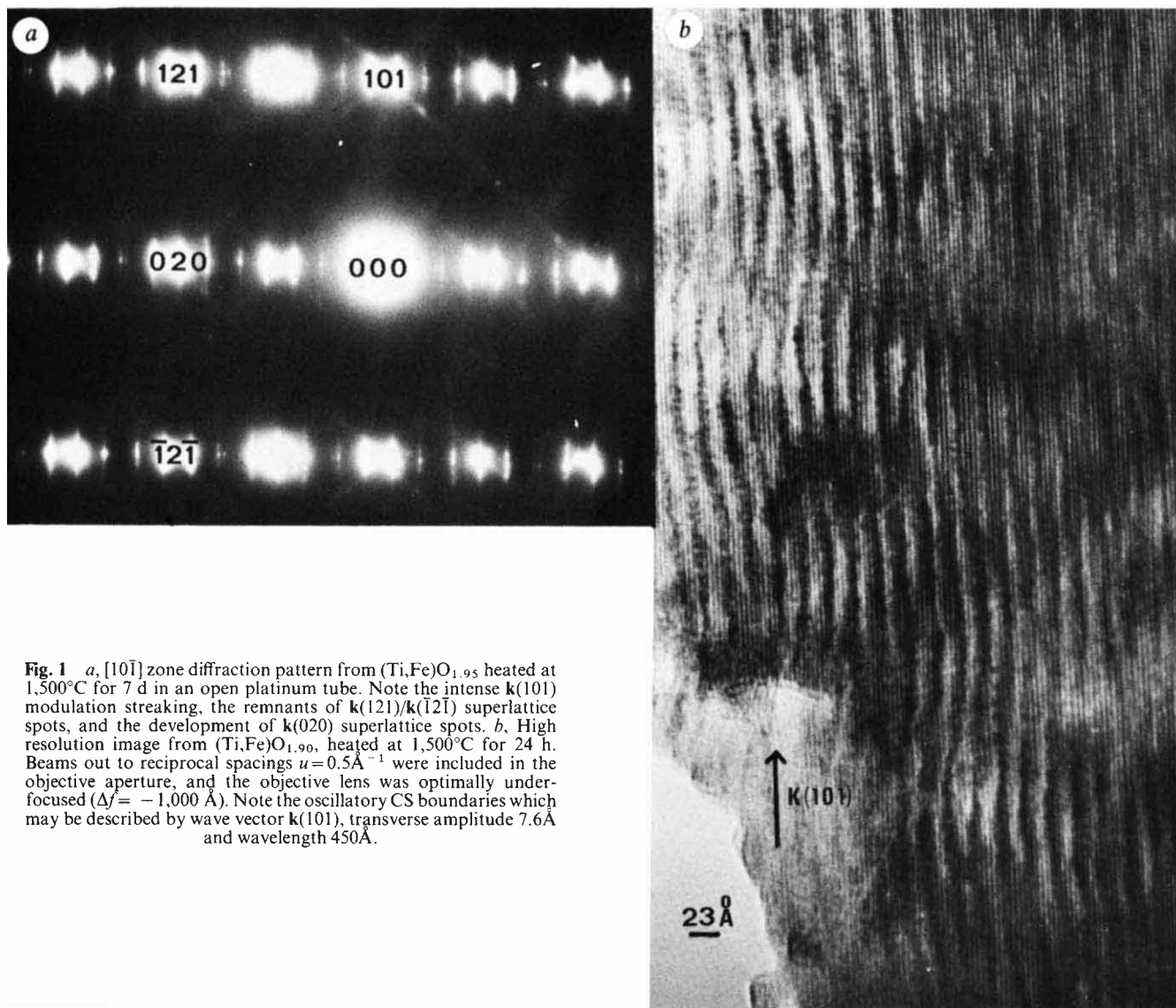


Fig. 1 *a*, $[10\bar{1}]$ zone diffraction pattern from $(\text{Ti,Fe})\text{O}_{1.95}$ heated at $1,500^\circ\text{C}$ for 7 d in an open platinum tube. Note the intense $k(101)$ modulation streaking, the remnants of $k(121)/k(\bar{1}2\bar{1})$ superlattice spots, and the development of $k(020)$ superlattice spots. *b*, High resolution image from $(\text{Ti,Fe})\text{O}_{1.95}$, heated at $1,500^\circ\text{C}$ for 24 h. Beams out to reciprocal spacings $u=0.5\text{\AA}^{-1}$ were included in the objective aperture, and the objective lens was optimally under-focused ($\Delta f=-1,000\text{\AA}$). Note the oscillatory CS boundaries which may be described by wave vector $k(101)$, transverse amplitude $7.6\text{\AA}$ and wavelength $450\text{\AA}$.

corresponding high-resolution image (Fig. 2*b*) again shows oscillatory CS with mean orientation (020). These are transverse composition waves propagating along $k(100)$ with amplitude $4.6\text{\AA}$ and wavelength $75\text{--}100\text{\AA}$. Different preparations showed a range of amplitude and wavelengths, paralleling the diffraction observations.

$k(001)$ modulated structures: Fig. 3*a* shows a $[100]$ zone diffraction pattern with modulation sidebands along $k(001)$ associated with the rutile sub-cell reflections. Fine satellite spots may be resolved (see inset, Fig. 3*a*) indicating $250\text{\AA}$ wavelength. The corresponding image (Fig. 3*b*) shows almost sinusoidal CS oscillations of boundaries that are mostly edge-on. Some darker areas resolved along the boundaries may indicate ribbon-like twisting out of the zone. Nevertheless, variations occur in a highly cooperative manner. This transverse composition wave along $k(001)$ has amplitude approximately $10\text{\AA}$ and wavelength $240\text{\AA}$, the CS indices ranging from $k(061)$ to $06\bar{1}$. Figure 3*c* shows a further example of almost perfect sinusoidal oscillations, of considerably larger amplitude (approximately $25\text{\AA}$), again having mean plane (020) but CS indices ranging from (041) to $(04\bar{1})$. The wavelength is $335\text{\AA}$.

High-temperature ordered ($0kl$) structures: examples of diffraction patterns and images showing long rows of sharp superlattice spots along $k(0kl/0k\bar{l})$ and characteristic zig-zag textures, with sharp coherent (001) interfaces are given in ref. 6. Some crystals show almost perfect $k(020)$ structures with straight, parallel CS boundaries over several $100\text{\AA}$.

Structural models and schematic reaction mechanisms

The low- to high-temperature transformation clearly involves cooperative reorientation of the CS indices from $\{hkl\}$ in $\langle 111 \rangle$ zones to $\{0kl\}$ in $\langle 100 \rangle$ zones. The high-temperature CS spacings are directly related to the $\{121\}$ CS spacings at low temperature, suggesting that changes in orientation occur by diffusion and/or rearrangement of existing boundaries without the necessity for new boundaries to be created by dislocation mechanisms. However, the nature of the structural elements and the metal ordering within the CS boundaries changes significantly during the transformation. The $(121)[0-\frac{1}{2}\frac{1}{2}]$ CS operation is known to produce corundum-like chains of edge- and face-shared chains along $[1\bar{1}1]$ (Fig. 4*a*). These also link to form corner- and face-shared chains along $[10\bar{1}]$. The corundum-type structure (as in Ti_2O_3 , Cr_2O_3 or Fe_2O_3) comprises identical $[10\bar{1}]$ chains (Fig. 4*b*) but these link to form (020) slabs by octahedral edge-sharing along $[102]$. The metal atom ordering within (020) CS boundaries comprises intergrowth of corundum- and rutile-type elements within slabs of finite width (Fig. 4*c*, shown edge-on in Fig. 1*b*). Within the corundum part of the intergrowth, viewed along $[012]$ in Fig. 4*d*, two interpenetrating sets of $[\bar{1}\bar{1}1]$ and $[1\bar{1}\bar{1}]$ edge-face-shared chains occur. These are now of finite length and confined to the (020) intergrowth slab. Identical but infinite chains occur in the $(\bar{1}21)$ and $(12\bar{1})$ low-temperature forms. Thus, during the phase transformation the integrity of the $[10\bar{1}]$ chains persists although

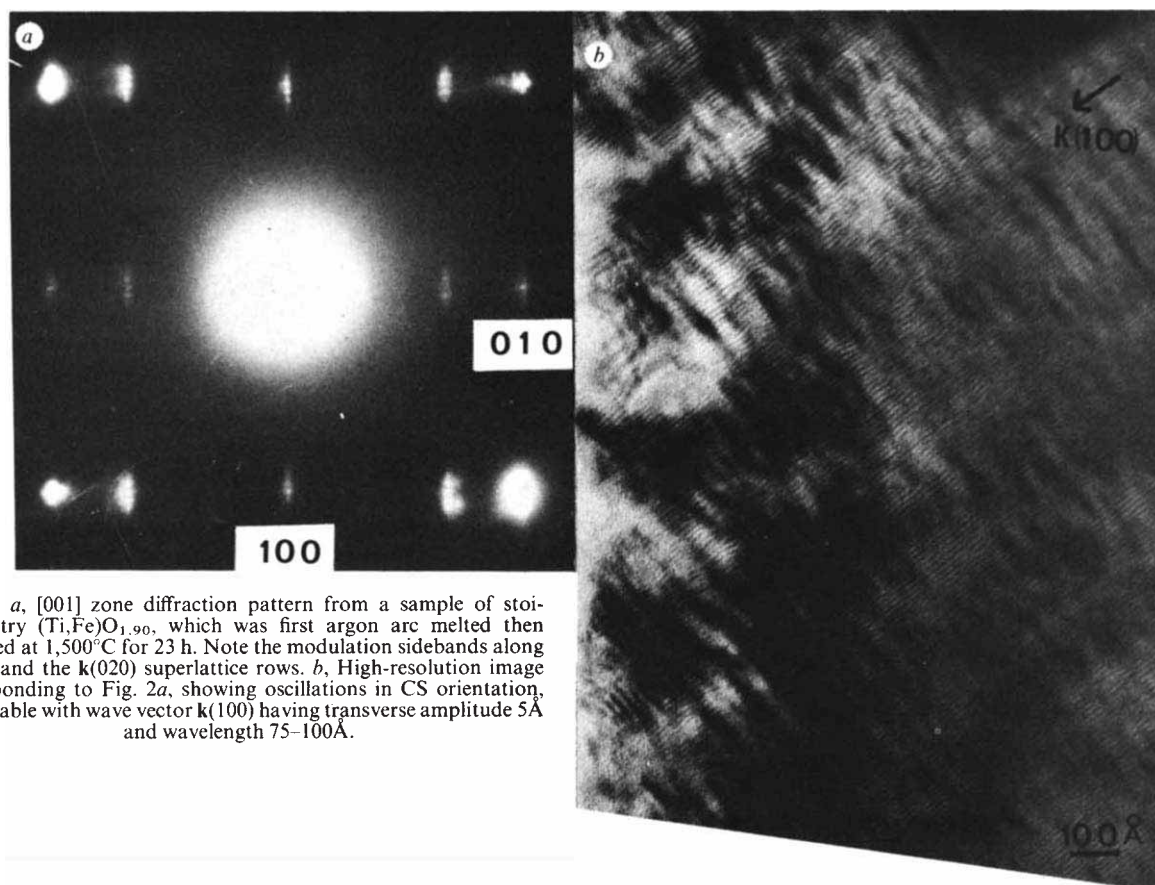


Fig. 2 *a*, [001] zone diffraction pattern from a sample of stoichiometry $(\text{Ti,Fe})\text{O}_{1.96}$, which was first argon arc melted then annealed at $1,500^\circ\text{C}$ for 23 h. Note the modulation sidebands along $k(100)$ and the $k(020)$ superlattice rows. *b*, High-resolution image corresponding to Fig. 2*a*, showing oscillations in CS orientation, describable with wave vector $k(100)$ having transverse amplitude 5Å and wavelength $75\text{--}100\text{Å}$.

these link together forming much larger corundum-like columns along $[10\bar{1}]$. The integrity of $[\bar{1}\bar{1}1]$ chains is lost but short lengths of $[\bar{1}\bar{1}1]$ and $[1\bar{1}\bar{1}]$ chains are incorporated into the high-temperature (020) structure.

Our high-resolution imaging and diffraction study³ shows that at temperatures approaching $1,450^\circ\text{C}$ (121) and $(\bar{1}\bar{2}\bar{1})$ CS domains intersect forming (101) interfaces containing the $[10\bar{1}]$ chains characteristic of both high- and low-temperature forms. These interfaces are not classical twin boundaries, as although the CS superstructure appears twinned, the rutile sub-cell is not. The (101) interfaces increase in number with increasing temperature until $k(121/\bar{1}\bar{2}\bar{1})$ structures zig-zag on the finest possible scale. This transformation explains the development and then decay of the $k(101)$ modulated structures. This reaction, occurring in the $[10\bar{1}]$ zone and involving (101) interfaces, may be summarised by the equation

$$(020) = (121) + (\bar{1}\bar{2}\bar{1}) \quad (3)$$

Similarly, the structural origin of $k(100)$ modulation lies in the combination of $k(121)$ and $k(\bar{1}\bar{2}\bar{1})$ low temperature forms through $k(100)$ domain interfaces. These also increase in frequency for temperatures approaching $1,450^\circ\text{C}$, providing an alternative path to high-temperature structures by

$$(021) = (121) + (\bar{1}\bar{2}\bar{1}) \quad (4)$$

and

$$(02\bar{1}) = (12\bar{1}) + (\bar{1}\bar{2}\bar{1}) \quad (5)$$

In these cases, the end members and any intermediate structures have structural elements with common zone axes $[01\bar{2}]$ and $[012]$. Reactions (4) and (5) can then be combined to produce (020) by

$$(020) = (021) + (02\bar{1}) \quad (6)$$

This would involve (001) modulations in the $[100]$ zone.

A third possible combination of low-temperature structures

involves (001) domain boundaries between $k(121)$ and $k(12\bar{1})$. This does not lead directly to an observed high-temperature structure on $(0kl)$, we obtain

$$(120) = (121) + (12\bar{1}) \quad (7)$$

and

$$(\bar{1}\bar{2}0) = (\bar{1}\bar{2}1) + (\bar{1}\bar{2}\bar{1}) \quad (8)$$

containing common structural elements along $[210]$ and $[2\bar{1}0]$ respectively. (It is interesting that the CS operation $(120) [\frac{1}{4} \frac{1}{2} \frac{1}{2}]$ has been observed in gallia-doped rutile, where elements of $\beta\text{-Ga}_2\text{O}_3$ intergrow coherently with rutile on (120) planes⁸. Similarly (010) $[\frac{1}{4} \frac{1}{2} \frac{1}{2}]$ boundaries have been observed in Mn-doped rutile (Stone and L.A.B., in preparation).)

One could then use $k(100)$ modulation waves to obtain (020) from equations (7) and (8) by

$$(020) = (120) + (\bar{1}\bar{2}0) \quad (9)$$

in the $[001]$ zone. There is some evidence for the more complex paths

$$(4) + (5) = (6)$$

and

$$(7) + (8) = (9)$$

but they appear to be less frequent than reaction (3) (ref. 3). Thus reactions (4), (5) and (9) account for the $k(100)$ modulated structures whereas reactions (7), (8) and (6) account for $k(001)$ modulations.

Further continuity can be realised in the transformation mechanism by writing reactions (3) to (9) in the form

$$(\quad) = p(\quad) + p'(\quad)$$

where p, p' are integers, and the ratio p/p' varied. This could lead to a ribbon-like twisting of CS boundaries.

Young's modulus plots, elastic strain energy and CS orientations in reduced rutiles

The likelihood that elastic strain energy may be an important factor in determining CS orientations is suggested by X-ray structure refinements of V_nO_{2n-1} (ref. 9). Two types of atomic displacements occur relative to the ideal CS operation

$(121)[0-\frac{1}{2}\frac{1}{2}]$. There are small local displacements due to metal-metal repulsion across face-shared octahedral pairs and to topological changes in the oxygen net. These types of displacements have been modelled in the theoretical analyses of Stoneham and Durham¹⁰ and also Iguchi and Tilley¹¹. However, superimposed on these local displacements there is an overall expansion

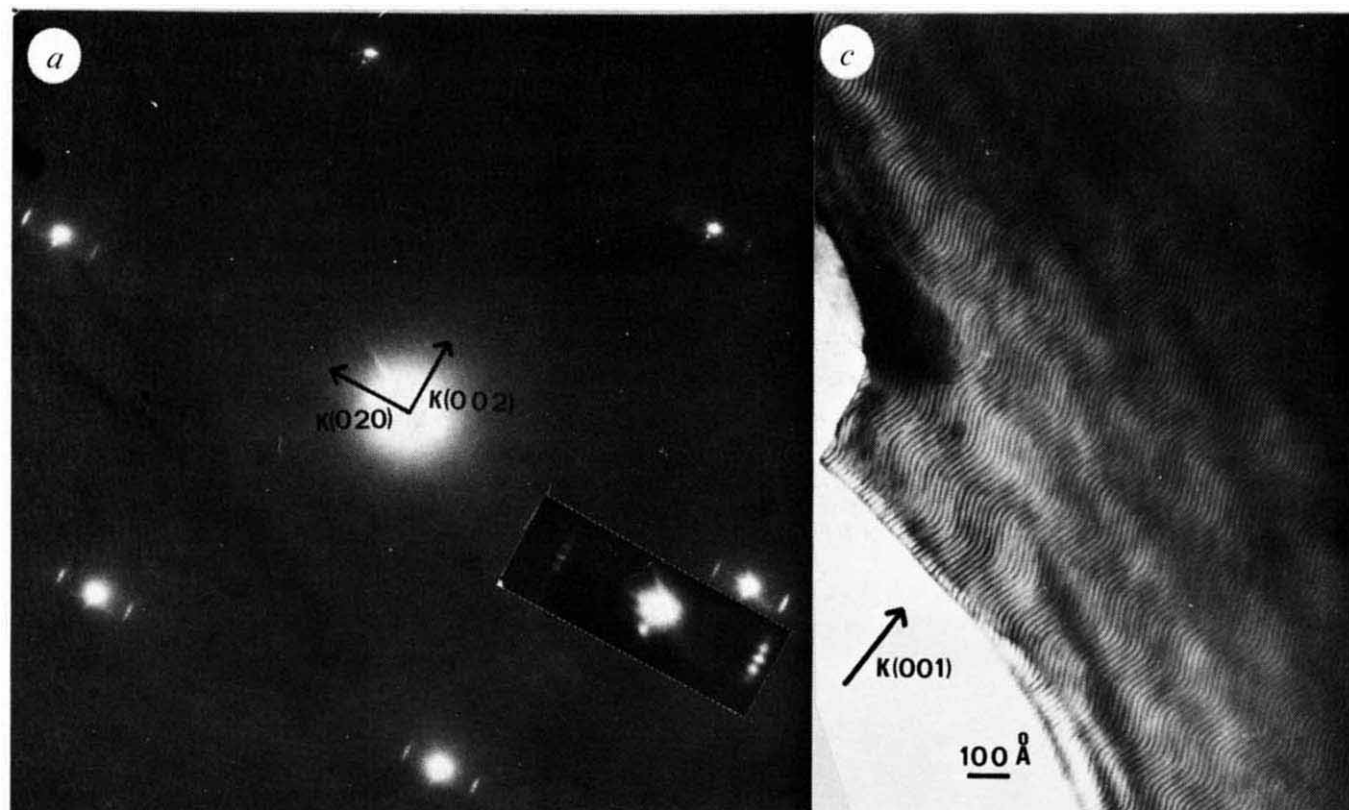


Fig 3 *a*, [100] zone diffraction pattern from $(Ti,Fe)O_{1.90}$ heated at 1,500°C for 24 h. Note $k(001)$ modulation sidebands with periodicity approximately $250\text{\AA}^{-1}$ (see inset). *b*, High-resolution image corresponding to *(a)* showing 23Å spacing wave-like CS structure. Note transverse modulation vector $k(001)$ with amplitude approximately $5\text{\AA}$ and wavelength 240Å. *c*, High resolution image of same preparation showing perfect sinusoidal CS waves oscillating from $k(041)$ to $k(041)$ with mean plane (020). Transverse modulations have amplitude 25Å and wavelength approximately 335Å.

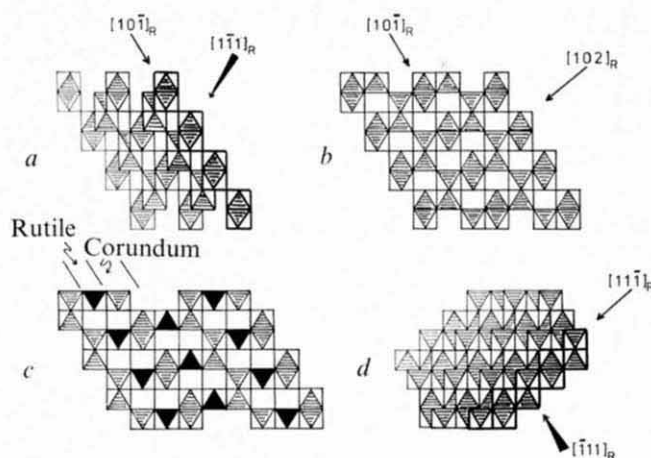


Fig. 4 *a*, Articulation of octahedra within a (121) CS boundary, viewed along [010], showing face-shared pairs of octahedra joined by edges into chains along $[111]$ and by corners into chains along $[101]$. *b*, Equivalent view of a layer of octahedra in corundum-type structure. *c*, [010] view of an octahedral layer showing intra-boundary metal atom ordering and 1:1 intergrowth of corundum and rutile parallel to $[101]$. *d*, (101) slice through the corundum-type structure, viewed along $[012]$. Note the interpenetrating $[111]$ and $[111]$ face-edge octahedral chains.

sion of the real structures relative to the ideal in direction normal to the CS boundary. For the V_nO_{2n-1} ($3 \leq n \leq 7$) family, real and ideal superlattice spacings are given by

$$D^r = d_{121}(n-0.35) \quad \text{and} \quad D = d_{121}(n-\frac{1}{2}) \quad (10)$$

A much smaller fractional change occurs for the two cell parameters lying within (121). We estimate the elastic strain contribution to the formation energy of CS structures to be

$$U_{\text{strain}} = \frac{1}{2}E\delta^2 \quad (11)$$

where E is Young's modulus for directions $\mathbf{k}(hkl)$ and δ is the fractional dilation of the real structure relative to the ideal. Values of approximately $5 \times 10^{-3} \text{ eV } \text{\AA}^{-2}$ were obtained. These are much less than typical stacking fault energies for metals ($\approx 5 \times 10^{-1} \text{ eV } \text{\AA}^{-2}$). This might have been anticipated as CS boundaries are almost always infinitely extended, and not limited by partial dislocations.

The remarkable relationship between the minima in Young's modulus plots and the observed orientations of CS boundaries, however, is the main point of this section. Values of Young's modulus were calculated using

$$E^{-1} = S_{11}(l^4 + m^4) + S_{33}n^4 + m^2l^2(2S_{12} + S_{66}) + n^2(l^2 + m^2)(2S_{13} + S_{44}) \quad (12)$$

where S_{ij} are the elastic compliance constants and l, m, n are the direction cosines. Elastic compliances were obtained from ref. 13. The Young's modulus plot for the $[1\bar{1}1]$ zone (Fig. 5a) shows a minimum exactly midway between $\mathbf{k}(121)$ and $\mathbf{k}(132)$ so that all the low-temperature structures vary by -7.2° to $+4.4^\circ$ from the minimum at $\mathbf{k}(253)$. Thus it seems that elastic strain is the dominant factor determining CS orientation, at least for bound-

Fig. 5 Plots of Young's modulus for *a*, $[1\bar{1}1]$; *b*, $[10\bar{1}]$; *c*, $[001]$; and *d*, $[100]$ zone axes. Note minimum for $[1\bar{1}1]$ lies exactly midway between $\mathbf{k}(121)$ and $\mathbf{k}(132)$ whereas absolute minima occur along $\mathbf{k}(010)$ and $\mathbf{k}(100)$. One division equals $10^{12} \text{ dyn cm}^{-2}$.

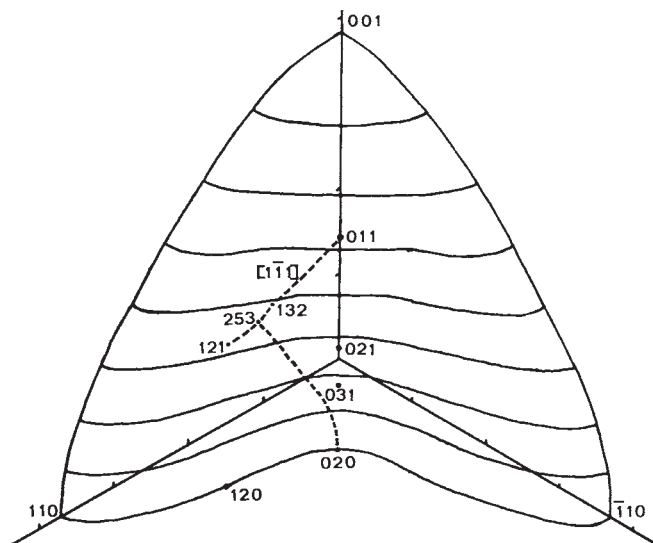
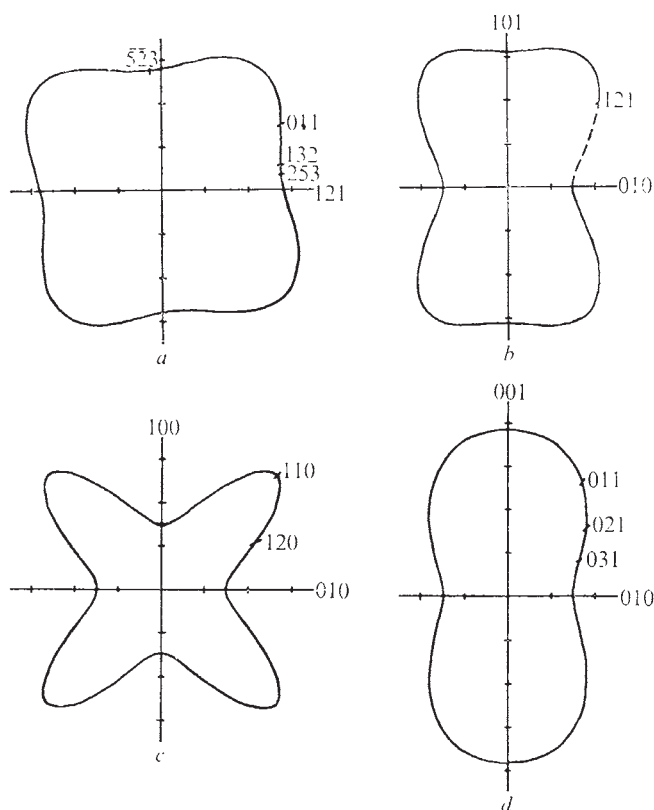


Fig. 6 Perspective view of section of three-dimensional Young's modulus plot. Note the dotted path from $\mathbf{k}(253)$ to $\mathbf{k}(020)$, representing the path of steepest descent from low- to high-temperature structures. One division equals $10^{12} \text{ dyn cm}^{-2}$.

daries constrained to be in the $[1\bar{1}1]$ zone. Symmetrically equivalent minima occur for the other $\{hkl\}$ types, see for example (523) in Fig. 5a.

The other significant Young's modulus plots are shown in Fig. 5b, c, d for the $[10\bar{1}]$, $[001]$ and $[100]$ zones. These all show a pronounced minimum at $\mathbf{k}(020)$. Construction of a three-dimensional model shows that $\mathbf{k}(020)$ is also the absolute minimum. Thus if CS is no longer constrained to lie in the $[1\bar{1}1]$ zone, we expect $\mathbf{k}(020)$ to be the equilibrium orientation. This presupposes that an appropriate structural mechanism exists and that a suitable reaction path can be found. Our observation that $\mathbf{k}(020)$ is precisely the orientation favoured by the high-temperature structures strongly supports the suggestion that elastic strain energy determines CS orientation. The observed modulation vectors $\mathbf{k}(101)$, $\mathbf{k}(100)$ and $\mathbf{k}(001)$ in the $[10\bar{1}]$, $[001]$ and $[100]$ zones, represent oscillations within the elastic strain energy potential well surrounding $\mathbf{k}(020)$. It is significant that the slope of the plots in the vicinity of $\mathbf{k}(020)$ increases in the order

$$[100] < [10\bar{1}] < [001] \quad (13)$$

Thus $\mathbf{k}(001)$ modulations would be expected to have larger amplitude than $\mathbf{k}(10\bar{1})$, which would in turn be greater than $\mathbf{k}(100)$. Experimentally we would expect that for increasing temperature, $\mathbf{k}(100)$ modulations would be eliminated first, then $\mathbf{k}(10\bar{1})$ and finally $\mathbf{k}(001)$.

Conclusion

Inspection of the three-dimensional model shows that the path of steepest descent from low- to high-temperature structures runs from $\mathbf{k}(253)$ to $\mathbf{k}(020)$, shown on Fig. 6. If the low-temperature structure starts at $\mathbf{k}(121)$, which is 7° away from $\mathbf{k}(253)$, then reaction (3) occurs, path dotted on Fig. 5b. Reactions (4)–(9) may be similarly represented. Reactions (3)–(9) have been observed experimentally but further long-annealing experiments will be necessary to determine equilibrium transformation paths.

It could be suggested that (121) structures are metastable with respect to (020) but we have observed^{1,3} that low-temperature structures are recovered on annealing high-temperature forms at temperatures $\leq 1,400^\circ\text{C}$, suggesting that the transformation is in fact reversible.

If elastic strain energy is the dominant factor determining CS orientations, it should be possible to induce preferred orientations to grow at the expense of other equivalent $\{hkl\}$ structures by applying uniaxial stresses to suitably doped rutile crystals. Orientations having elastic strain components parallel to the applied

stress should be eliminated, increasing sample homogeneity and grain size. Experiments designed to apply 10–1,000 atm normal to $[1\bar{1}1]$ slices are in preparation.

The above experimental and theoretical work raises further questions. Clearly elastic constant measurements are required for a wide range of materials subject to CS, especially the tungsten oxides. Other enthalpic and entropic factors contributing to the free energy and determining the fine detail involved in the temperature and stoichiometry dependence of CS orientations and spacings remain to be identified and studied.

It is tempting to suggest that for temperatures very close to the melting point ($\geq 1,550^\circ\text{C}$) the modulations of the type illustrated in Figs 1–3 may become dynamic standing or even travelling waves. Simple diffusion mechanisms may be derived to allow this to happen. The CS spacings would remain essentially constant but the orientations would vary cooperatively. Perhaps such a dynamic situation is premonitory to melting.

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Nucleotide sequence of *cro*, *cII* and part of the *O* gene in phage λ DNA

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*A nucleotide sequence comprising 960 base pairs of bacteriophage λ DNA has been determined. The sequence includes the entire genes of the regulatory proteins *cro* and *cII*, and part of the *O* gene, together with control elements for their transcription and translation. The right-hand boundaries of the λ imm434 and λ imm21 substitutions and the *cy42* mutation have been located.*

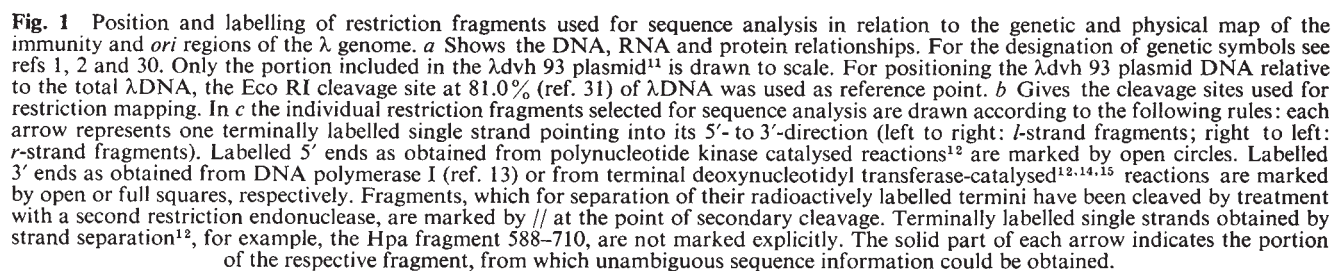
DURING the life cycle of bacteriophage λ , either production of progeny phage followed by lysis of the host cell or integration of phage DNA into the host chromosomal DNA resulting in a lysogenic cell can occur as the two genetically determined alternative programmes^{1,2}. For this reason, the expression of the λ genome can be regarded as a model for the genetically determined differentiation processes occurring in cells of higher organisms. The initiation of the lytic pathway is largely governed by the promoters P_R and P_L (controlled by the operators O_R and O_L , respectively). Immediately after infection, they direct the production of two short messenger RNAs coding for the proteins *cro* and *N*, respectively. Mediated by the anti-terminating activity of the *N*-protein, the two messages are then extended beyond their factor ρ -dependent³ sites t_{R1} and t_{L1} , followed by production of the proteins *cII*, *O*, *P* and *cIII*. For initiation of the lysogenic pathway, synthesis of the repressor protein *cI* has to be established. It is still uncertain whether the establishment mode of *cI* transcription, which is positively regulated by the proteins *cII* and *cIII* (refs 4–6), originates at the promoter: P_O (ref. 7) or at a promoter P_{re} defined by *cis*-dominant mutations located in the *cy* region^{8,10}. After installation of lysogeny, production of the *cI* message is maintained at lower rates by the activity of the promoter P_{rm} , which is positioned directly to the right of the *cI* gene⁸. In order to provide a physical basis for understanding these mechanisms and their underlying molecular structures in more detail, we have under-

taken to sequence the segment of λ DNA that extends from the P_R promoter into gene *O*.

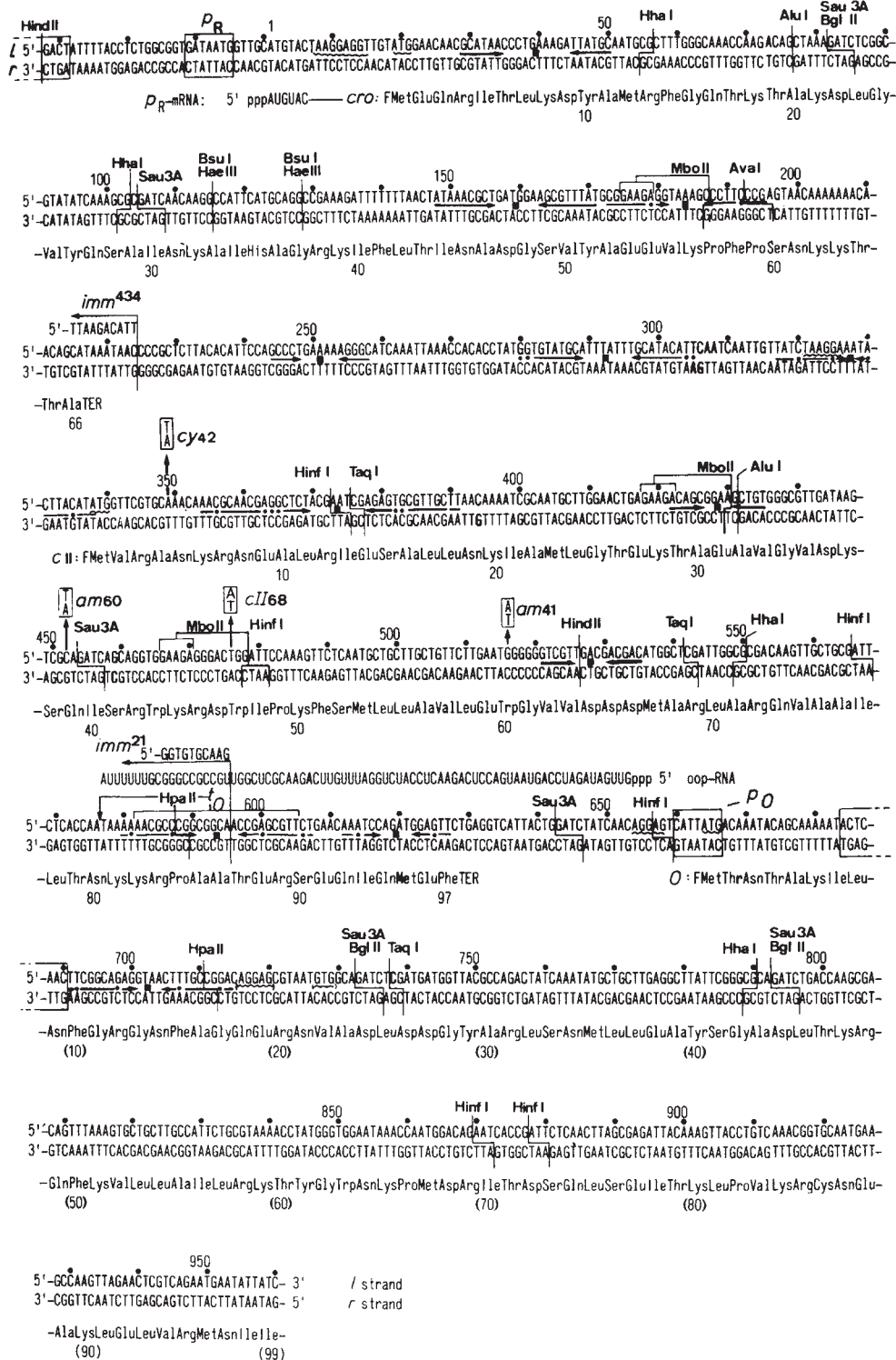
Sequence determination

In order to deal with a smaller number of restriction fragments, DNA of the small λ -derived plasmid λ dvh 93 (ref. 11) was used for most of the experiments instead of DNA from the whole λ phage (Fig. 1a). However, extensive portions of the DNA sequence analysis have been repeated using bacteriophage DNA fragments obtained from various sources such as λ cIIam60am41, λ cy42 and others, and no differences have been detected except for those that represent the respective phage mutations. For localisation of the λ imm21 and λ imm434 right hand homology boundaries, λ dv strains carrying the respective hybrid immunity regions were constructed (K. Matsubara, unpublished, and G.H., unpublished) and used for isolating the appropriate restriction fragments.

After constructing tentative physical maps of the λ dv plasmid DNA fragments obtained by the action of the various restriction endonucleases listed in Fig. 1b, selected DNA fragments were terminally labelled with ³²P-phosphate at their 5' or 3' ends (Fig. 1c), and sequenced using the dimethylsulphate/hydrazine technique¹². 5'-terminal labels were introduced by using polynucleotide kinase and [γ -³²P]rATP¹² (Fig. 3a). 3'-Terminal labelling was achieved by using either DNA polymerase I and [α -³²P]dATP¹³ or terminal deoxynucleotidyl transferase and [α -³²P]rATP^{12,14,15}. The DNA polymerase I reactions resulted in very homogeneous sequence patterns (see, for example, Fig. 3b). In contrast, the fragments labelled by use of the terminal transferase led to ghost bands in the sequencing gel, one nucleotide position below the main bands (see Fig. 3c). These bands, which are derived from the monoaddition product¹⁵, however, do not interfere with an accurate reading of the sequence. The 3'-labelling methods shown here complement the standard 5'-labelling method using polynucleotide kinase, so that any DNA fragment can be sequenced from both the



Termination of the cII protein is signalled by an UGA codon (629–631), which has been demonstrated to be a leaky terminator with wild-type ribosomes²⁰. For this reason a certain amount of readthrough up to the next in-phase terminator codon (UGA at position 665–667) may be anticipated, similar to the readthrough observed for the protein O (ref. 20). The λ cII68 missense mutation²² is a transversion at position 476 and causes an exchange of tryptophan to arginine at position 47 of the cII protein sequence, as deduced in Fig. 2. The transversion observed for the λ cY42 mutation²² at position 349 (Fig. 2) lies within the DNA sequence coding for the N-terminal part of protein cII. As this mutation is located at the third position of



an alanine codon, it will not alter the properties of the cII protein. Thus, the cII gene does overlap with the two DNA signals t_O and cy and with DNA sequence coding for the 3'-terminal two-thirds of oop RNA. Furthermore, it extends to the right of the λ imm21 boundary. As the cII function is dispensable for λ dv plasmids²³, the entire cII gene sequence has been confirmed by using restriction fragments derived from phage λ DNA.

The position of the correct initiator codon for the translation of protein O in the single remaining reading frame has to be to the left of position 793, as a deletion of the Bgl II fragment 733–792 will abolish O function in λ -derived plasmids (tested *in vivo*, M. Lusky and G.H., unpublished observation). This

leads to the AUG codon at position 664–666 or to the GUG codon at position 727–729, as both are preceded by very similar sequences complementary to the 3'-terminus of ribosomal 16S RNA. At present, we only tentatively favour the AUG codon 664–666, as a GUG codon has been identified as a natural initiator codon only twice²⁴. According to the reported molecular weight of 34,500 for the O protein²⁰, the 99 N-terminal amino acids shown in Fig. 2 would represent about one-third of the entire protein.

Concluding remarks

The amino acid compositions of all three proteins reveal relatively high amounts of polar, and especially of basic, amino

acids. Whether the rhythmic spacings of the latter of 4 to 5 positions apart that are noticeable in several regions reflect stretches involved in interaction with DNA remains to be seen. A detailed discussion of the controlling elements which govern the transcription of the *cro/cII/O* region will be presented elsewhere. We wish, however, to summarise the following points: (a) a comparison of the DNA sequences at the known termination sites of this region of λ DNA (for example, the λ - t_O site, see Fig. 2) and of analogous regions of the other lambdoid phage DNAs (such as the λ imm 21- t_O or Φ 80- t_O sites, unpublished observations) with termination sites occurring in other

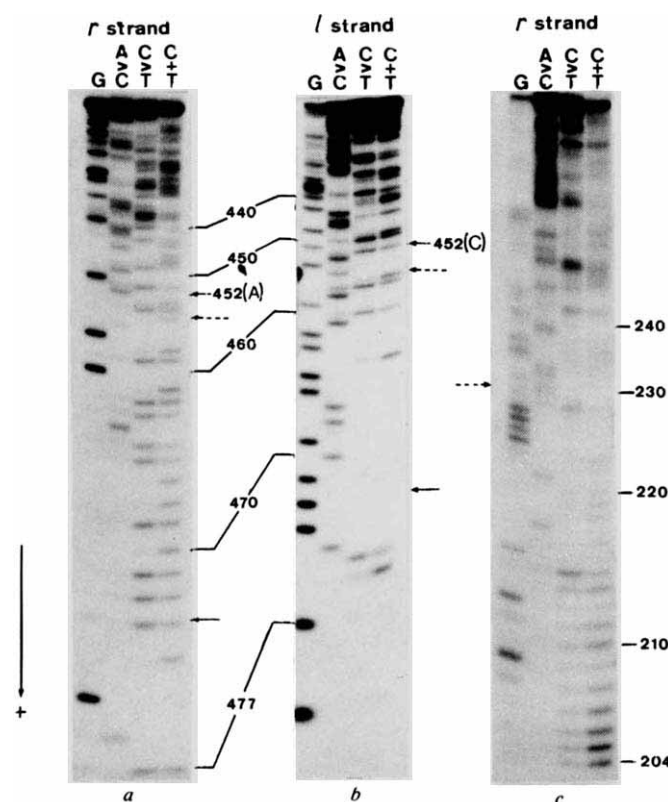


Fig. 3 Autoradiographic pictures of electrophoretically separated product mixtures obtained after chemical degradation¹² of restriction fragments labelled by reactions catalysed by polynucleotide kinase (a), DNA polymerase I (b), and terminal deoxynucleotidyl transferase (c). The nos beside the autoradiograms refer to the corresponding positions in the sequence shown in Fig. 2. The solid and dashed arrows indicate the positions of the bromphenol blue and of the xylene cyanol FF markers, respectively. The columns labelled by G, A > C, C > T, and C+T show the products of the corresponding reactions. a Shows the patterns obtained from the 5'-terminally labelled *Hinf*I fragment 376-481 (*r*-strand positions) derived from DNA of the λ cIIam60am41 mutant phage. This fragment carries the cIIam60 transition (A in position 452). b Shows patterns from which the complementary *l*-strand region of the same *Hinf*I fragment 376-481 can be read from wild-type phage λ DNA. This fragment had been labelled 3'-terminally by a DNA polymerase I-catalysed reaction: about 5 pmol of the isolated *Hinf*I fragment were incubated with 20 pmol of [α -³²P]dATP (250 Ci mmol⁻¹; Amersham), 2 nmol of dGTP and 12 units (54 ng) of homogeneous DNA polymerase I (ref. 38) from *Escherichia coli* in the presence of 20 mM Tris-HCl, pH 7.5, 10 mM MgCl₂ and 0.1 mM DTE for 5 min at 25 °C in a total volume of 20 μ l. Further study was as usual¹². The C corresponding to wild-type *l*-strand position 452 (Fig. 2) is marked; it represents the only position where this sequence deviates from complementarity with the mutant sequence deduced from the pattern of (a). c Shows sequence patterns obtained from the wild-type λ dvh 93 plasmid DNA-derived *Mbo*II fragment 188-430, which had been labelled by a terminal deoxynucleotidyl transferase-catalysed reaction as described¹², using [α -³²P]rATP (250 Ci mmol⁻¹; Amersham). The degradation products were loaded on 40 $\times$ 22 $\times$ 0.2-cm slab gels of 20% polyacrylamide/7M urea (a and b) or 12% polyacrylamide/7M urea (c) and electrophoresed for 6 h at 1,000 V (a and b) and 5 h at 900 V (c).

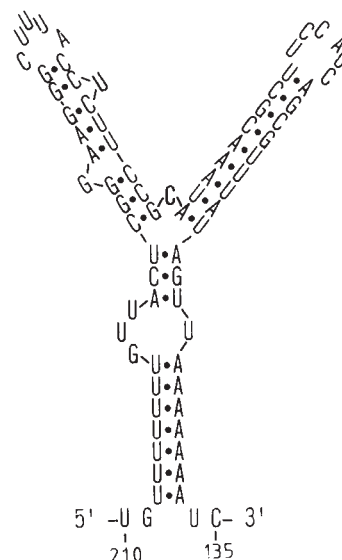


Fig. 4 A triple-palindromic segment of a hypothetical structure of a P_O or P_{Re} directed RNA. The complementary sequence, which occurs in the *cro* mRNA, can be folded into a similar structure (not shown).

DNA²⁵⁻²⁸ shows the presence of hyphenated palindromes as part of GC-rich sections²⁹ immediately preceding the final AT-rich tail sequences. These palindromes potentially fold up into hairpin loops of similar size. While some of these loops have been assumed earlier²⁵⁻²⁸ to occur at the mRNA level, we favour the possibility that they will fold up within RNA polymerase in the noncoding DNA strand during the process of transcriptional termination, effectively inhibiting the further progression of the enzyme. (b) The *cy42* mutation can be interpreted to involve either an *l*-strand terminator site (increasing its strength) or the recognition site of an *l*-strand promoter region; more *cy* mutant sequences remain to be determined to decide between these alternatives. (c) In addition to the palindromes related to controlling elements well known from genetic analysis, others have been observed by computer-aided analysis of the DNA sequence (see Fig. 2). While some of them also might be active as signals on the DNA level, we feel that they are much more likely to be implicated in RNA secondary structure(s). Taking into account stretches of complementary sequences further apart, it is possible to construct almost completely folded (tree-like) hypothetical RNA secondary structures covering positions 1 to 239. These may form in the untranslated P_O or P_{Re} directed RNA rather than in the ribosome-attached *cro* mRNA. In this hypothetical RNA fold-up, from which a small section is depicted in Fig. 4, the sequences 1-21/239-215 would hybridise at its stem and several branches would be displayed above.

Since the submission of this paper, the amino acid sequence of the *cro* protein has been published³⁹, and the nucleotide sequence of the *cro* gene has been determined independently⁴⁰.

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The relationship between function and DNA sequence in an intercistronic regulatory region in phage λ

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ρ factor-mediated transcription termination at the t_{R1} terminator site of bacteriophage λ is examined. Mutations affecting the termination event are characterised. These mutations define features of the site which seem to be important to terminator function. In addition, other related transcriptional and translational regulatory elements are defined within the region surrounding the termination site. The potential molecular interactions and structural overlaps of these control signals apparently couple the regulation of the decision between lytic and lysogenic growth patterns by phage λ .

TRANSCRIPTIONAL termination plays a major part in both the quantitative and temporal regulation of expression of the bacteriophage λ genome^{1–3}. Following phage infection or induction, the host RNA polymerase transcribes the 'early' genes of λ initiating from two major promoters, P_L and P_R , within the immunity region (Fig. 1). The majority of this transcription, both rightward and leftward, is limited initially to the immunity region by sites of transcription termination, t_L and t_{R1} , respectively (Fig. 1). Efficient transcription distal to these termination sites is dependent on expression of lambda N function, the gene product encoded by the P_L -initiated 'early' leftward transcript^{4–7}. Although the mechanism of action of N is not understood, N is thought to regulate transcription positively by allowing the RNA polymerase to read through the early termination sites (refs 8, 9 and our unpublished results).

In vitro, transcription of λ DNA by purified *Escherichia coli* RNA polymerase initiates at the same promoters and results in the production of relatively high molecular weight ($> 16S$) RNA transcripts which extend well beyond the immunity region and thus beyond the proposed transcription termination sites t_L and t_{R1} (ref. 8). Roberts^{8,10} isolated a host-encoded protein factor, ρ , which, when added to the *in vitro* transcription system,

resulted in termination of the P_L - and P_R -initiated transcripts and production of lower molecular weight products of defined size (as determined by sucrose gradient analysis). A 12S RNA product presumably encoding the N function is obtained from the left (l strand template) and a smaller RNA which encodes the cro gene product is obtained from the right (r strand template). These products are also observed as discrete 12S and 8–9S RNAs by electrophoretic separation on polyacrylamide

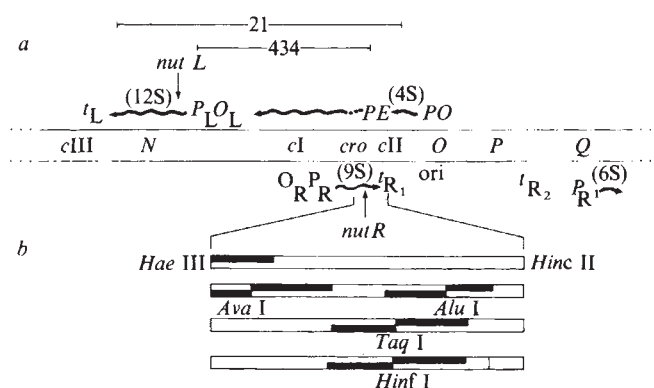


Fig. 1 Genetic map of bacteriophage λ showing the location of the cro - cII intercistronic boundary (the Y region) on the λ chromosome (a). Locations and relative sizes of the major RNA transcripts and the boundaries of the $\lambda imm434$ and $\lambda imm21$ immunity substitution regions are indicated. b An enlargement of a 416-base pair *HaeIII*-*HincII* DNA restriction fragment which contains parts of the cro (ref. 12) and cII genes and spans the intercistronic region between them. Other sites of restriction endonuclease cleavage are shown. The thickened black lines indicate those fragments which were 5'-end-labelled⁵⁷ and sequenced by direct DNA techniques¹⁸. See Fig. 2 for positions of restriction endonuclease cleavage sites within the sequence: *AvaI* (CCCGAG), positions 192–197; *HinfI* (GAATC), positions 372–376; *TaqI* (TCGA), positions 375–378; *AluI* (AGCT), positions 430–433.

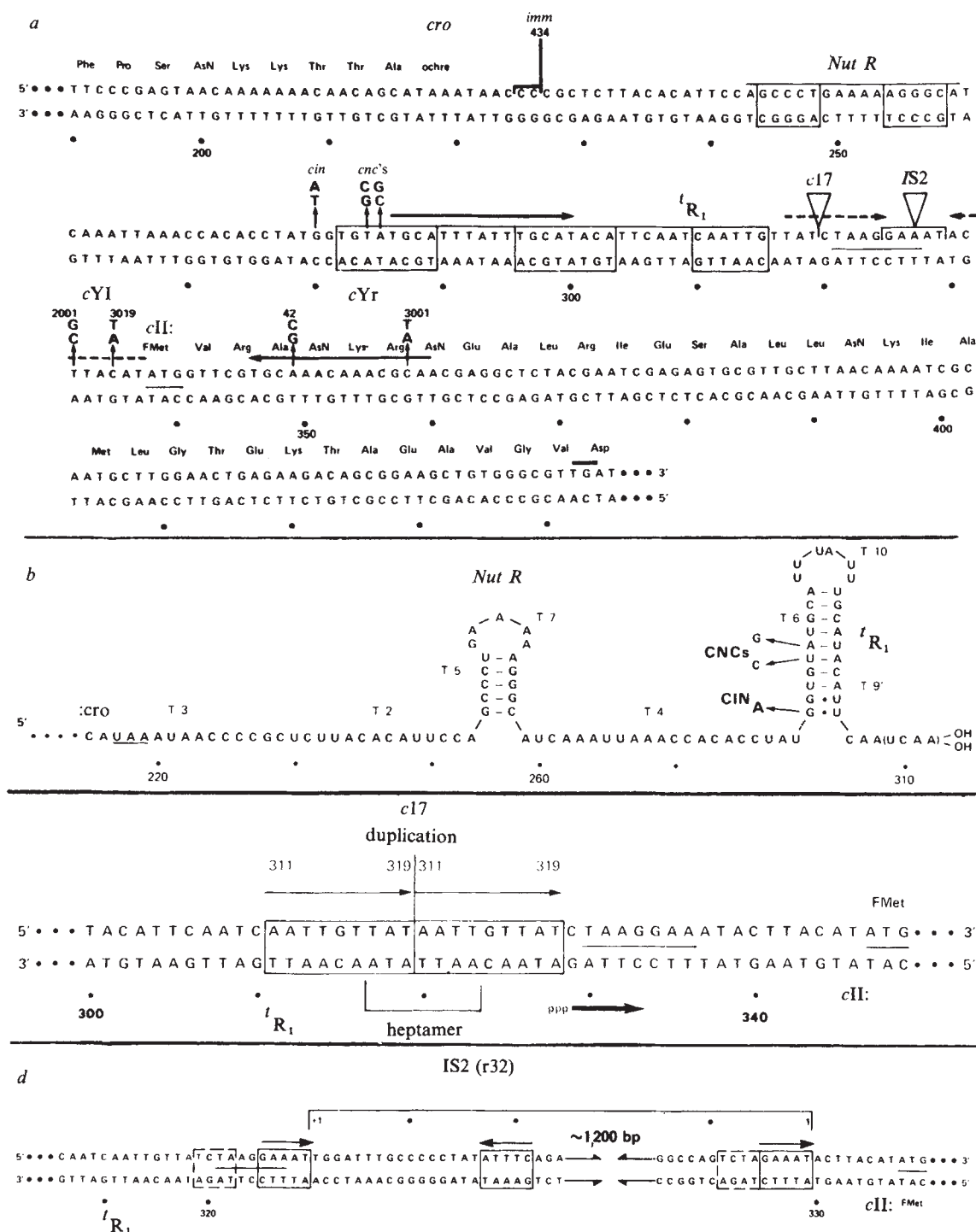


Fig. 2 *a*, DNA sequence of the intercistronic region between the λ *cro* and *cII* genes including the sequence in a variety of known mutants ($\uparrow$) within the region (see text for details). Residue numbers indicate relative position from the startpoint of transcription of the P_R initiated mRNA. Only those regions of dyad symmetry (that is, inverted repeat sequences) pertinent to the discussion in the text are shown. These occur at positions 244–248 and 258–254; 282–289 and 303–396; 286–299 and 359–346; 317–324 and 338–329; and are denoted either with boxes or horizontal arrows. Underscored residues at positions 321–327, 338–340 indicate the presumptive ribosome-binding site and AUG startpoint of the *cII* protein (see text). The mRNA transcript of this region exhibits the necessary complementarity to the 3' end of the *E. coli* 16S rRNA (refs 58, 59). In addition, sequence determination of several point mutations affecting *cII* function support our positioning of the *cII* coding region (unpublished). The right-hand boundary of the 434 immunity region substitutions (Fig. 1) is located to the right of the *cro* gene between residues 224–227. *b*, The primary and possible secondary structure at the 3' end of the *t*R₁ terminated mRNA. Mutations affecting termination are shown (see text for details). Residue positions are numbered as in (*a*). *T*₁ ribonuclease products are those indicated in Fig. 4*b*. *T*_{9'} represents the heterogeneous 3'-terminal oligonucleotides 9a, b, c, d, e from Fig. 3*b* and *c*. The secondary structure suggested is based on both thermodynamic considerations^{60,61} and relative susceptibility of the structure to partial enzymatic cleavage (our unpublished work). The underscored UAA sequence denotes the ochre termination signal of the *cro* gene¹². *c*, DNA sequence of the *c17* mutation of phage λ . Note that residue numbers indicated below the sequence differ from those in (*a*) beyond the site of the mutation in order to compensate for the additional 9 base-pair duplication. The startpoint and direction of transcription of the *c17*-initiated mRNA is indicated (see text for details). All other designations are identical to those shown in (*a*). *d*, DNA sequence surrounding the site of IS2 insertion in phage λ r32. The inserted DNA segment (that is, all nucleotide residues not occurring in the wild-type sequence shown in (*a*)) is designated by brackets (—). Residue numbers which occur below the sequence are identical to those in (*a*); those above the sequence refer only to the inserted DNA segment (+1 to -1). Arrows and boxes indicate both direct and inverted sequence repeats. All other designations are the same as those shown in (*a*).

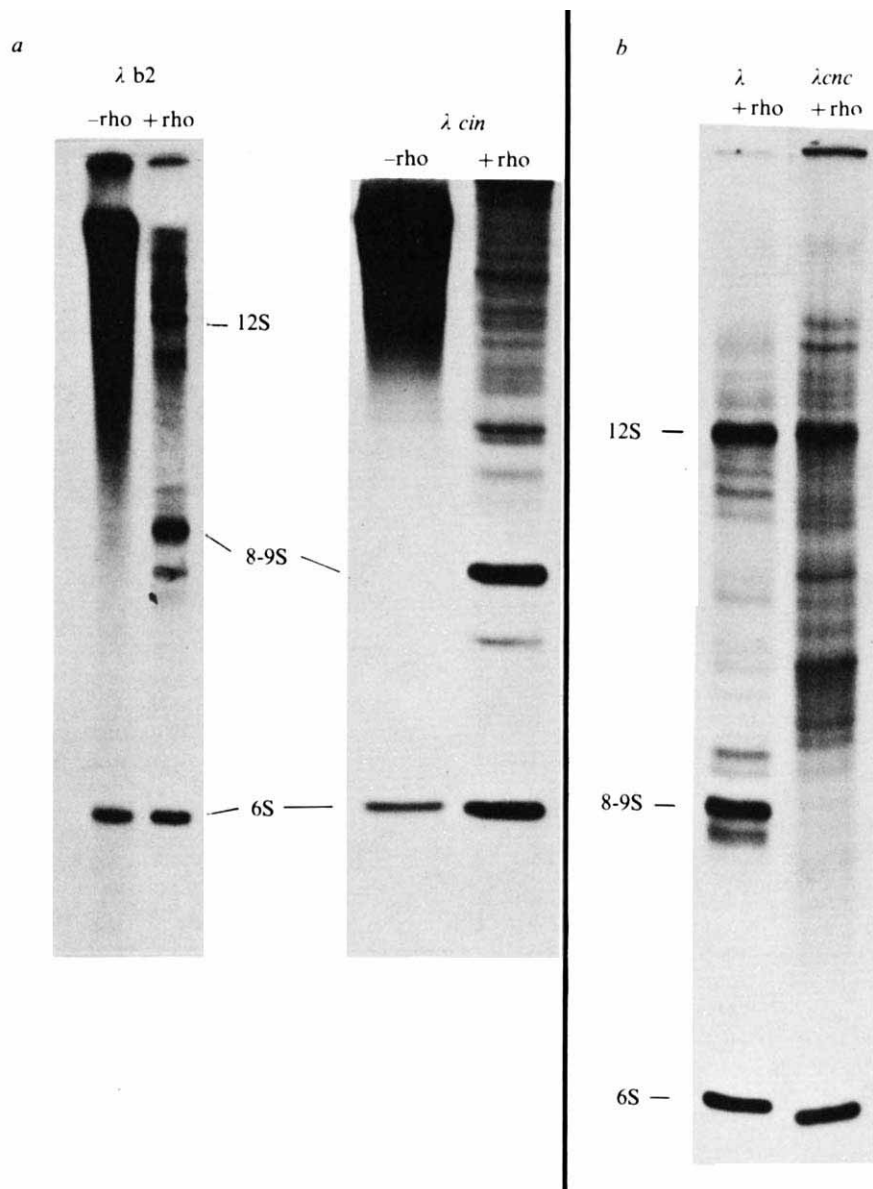


Fig. 3 Polyacrylamide gel electrophoresis of ³²P-labelled RNA synthesised *in vitro* from several λ DNA templates either in the presence or absence of ρ factor. *a*, λ b2S7 (λ b2) and λ c1857cin1S7 (λ cin); *b*, λ c1857S7 (λ) and λ c1857cin1cnc1S7 (λ cnc). DNAs were transcribed and analysed on 3.5% acrylamide slab gels which contained 8.0 M urea as previously described^{11,12}. The b2 region of λ accounts for ~50% of the transcription observed *in vitro*⁸. Thus, the templates used which contain the b2 region (for example, λ , λ cin, λ cnc) have additional transcription products not present in the analysis of the λ b2 RNA.

gels¹¹. From these results it has been inferred that the termination events observed *in vitro* to be ρ factor-dependent might be identical to those responsible for the synthesis of the 'early' mRNAs found in infected cells. This suggests that termination *in vivo* at t_{R1} requires the bacterial ρ factor and that the λ *N* protein counteracts this ρ -dependent termination (that is, transcriptional attenuation).

We have studied regulation, both *in vivo* and *in vitro*, of the ρ -terminated rightward mRNA transcript in the region surrounding the termination site, t_{R1} . We report here the nucleotide sequence of the intercistronic region between the λ *cro* and *cII* genes and define the exact site at which transcription from P_R terminates, both *in vitro* and *in vivo*. Several point mutants affecting termination at this site are characterised, as well as a number of other transcriptional and translational control signals which affect expression within this region. The structure of the termination site and its position relative to the other regulating elements which we have defined throughout this region are discussed. Certain of the results summarised and discussed here will be described in detail and published elsewhere.

Determination of the sequence

A combination of RNA and DNA sequencing techniques were used to determine an unambiguous nucleotide sequence (Fig. 2) for the regulatory region of phage λ which surrounds and includes the intercistronic region between the *cro* and *cII* genes

(Y region, Fig. 1). Identical techniques were also used to determine the sequence changes which occur within this portion of the genome in a variety of mutant phages altered in their ability to regulate properly transcriptional and/or translational expression (Fig. 2). The nucleotide sequence of the entire *cro* gene, which is immediately proximal to this region, has been reported elsewhere¹².

DNA (wild-type or mutant) was transcribed *in vitro* with purified *E. coli* RNA polymerase, either in the presence or absence of ρ factor, and the products fractionated on polyacrylamide slab gels as described previously¹¹ (Fig. 3). A discrete RNA transcript (8-9S), which is made only in the presence of ρ (Fig. 3a), was isolated and identified by hybridisation and fingerprint (Fig. 4a) analyses as the P_R -initiated early rightward mRNA transcript^{11,12} (that is, the *cro* gene message). This RNA was either used directly for sequence analysis or a fragment of this transcript containing only the 3'-terminal region (~100 nucleotides in chain length) was isolated by a single-step hybridisation to DNA obtained from the appropriate deletion-substitution mutant of λ (Fig. 4b). Application of standard RNA sequencing techniques¹³⁻¹⁵ allowed deduction of a unique sequence for the entire 3'-terminal region of the 8-9S RNA (Fig. 2b).

RNA sequence information for the region distal to the 3' end of the 8-9S RNA transcript was obtained from transcripts prepared *in vitro* in the absence of ρ factor. Appropriate hybridisation (Fig. 4d) of total transcript without previous gel

fractionation resulted in the selective isolation of RNA fragments which span the entire intercistronic region between *cro* and *cII* and extend into the *cII* gene. Again, standard RNA sequence analysis was used to characterise the region distal to the ρ -dependent termination site.

Direct DNA sequence analysis was carried out using the chemical procedures described by Maxam and Gilbert¹⁶. DNA restriction fragments obtained from either wild-type or mutant phage DNAs were end-labelled with ³²P and sequenced as depicted in Fig. 1. Autoradiograms of typical gels from which DNA sequences were deduced are shown in Fig. 5.

The RNA and DNA sequencing methods gave completely consistent results. Although each generates certain sequence ambiguities, in combination these techniques allow deduction of an unambiguous sequence with a level of accuracy much greater than that obtained by either method alone.

ρ -mediated transcription termination at t_{R1}

In vitro, transcription termination of the P_R -initiated mRNA (8–9S RNA) within the intercistronic region between the genes for *cro* and *cII* is absolutely dependent on the protein-termination factor ρ (Fig. 3a). In order to position the exact site on the DNA at which the ρ -dependent termination event occurs, the 3'-terminal oligonucleotide products produced from T₁ RNase fragmentation of the 8–9S RNA were selectively isolated by chromatography on dihydroxyboryl (DBAE) cellulose¹⁷. The 3' end fragments obtained by this procedure were initially characterised by two-dimensional homochromatography (Fig. 4c). Subsequent analyses of each separately resolved oligonucleotide indicated that they are all sequence related and differ only by the nucleoside residues occurring at their 3' termini. These same terminal oligos are detected in the T₁ fingerprint of the 100-nucleotide-long RNA fragment isolated by direct hybridisation from the 3'-terminal region of the 8–9S RNA (Fig. 4b, nos 9a, b, c, d, e). If, however, transcription

is carried out in the absence of ρ and the P_R -initiated mRNA is isolated and fingerprinted (as in Fig. 4d), these 3'-terminal oligonucleotides are not detected. All other T₁ products characteristic of the 3'-terminal region of the ρ -terminated transcript can be observed in this fingerprint, as well as additional new oligonucleotides which are not present in the 8–9S mRNA transcript. These new oligos represent transcription products derived from the region beyond the t_{R1} site of termination. One of these (oligo no. 9, Fig. 4d) has the sequence CAUACAUCUCAAUUG and is the only oligonucleotide consistent with transcriptional 'read-through' of the termination site. Thus, *in vitro*, it seems that ρ -mediated transcription termination in the t_{R1} site occurs heterogeneously over four or five adjacent nucleotide pairs and results in production of a 308–312-nucleotide-long mRNA, which includes 198 nucleotides that encode the 66 amino acids of the *cro* protein^{12,18}, the 18-nucleotide leader sequence for the *cro* message¹⁹, and 92–96 nucleotide residues (apparently untranslated) beyond the ochre translation termination triplet for *cro*. In the absence of ρ , the RNA polymerase 'reads-through' the termination site and into the *cII* gene to produce a single, contiguous polycistronic RNA transcript of high molecular weight.

We have used similar hybridisation and fingerprinting methods to monitor *r* strand transcription throughout the Y region in a variety of *in vivo* and *in vitro* conditions (our unpublished work). For example, in order to measure the efficiency of transcription termination at t_{R1} , *in vitro* reactions were carried out in the presence of rifampicin, in order to allow only a single round of transcription to initiate from the P_R promoter. This RNA was then selectively isolated by hybridisation and T₁ fingerprints prepared. Certain T₁ oligonucleotide products are uniquely characteristic for specific regions of the transcript, and their relative intensities are a measure of the amount of transcription in these regions. By determining the molar ratios of the appropriate T₁ products, we could compare directly

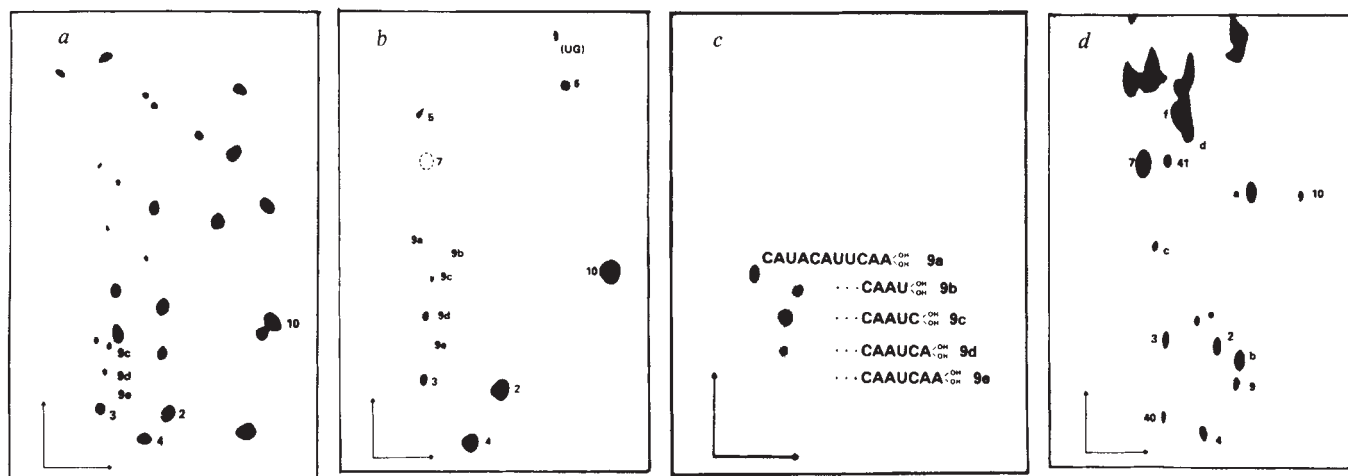


Fig. 4 Autoradiographs of two-dimensional fingerprints (prepared as previously described^{11,13-15}) of RNase T₁ oligonucleotide products derived from the P_R -initiated, rightward mRNA transcript. Horizontal dimension: electrophoresis on Cologel in 8.0 M urea at pH 3.5. Vertical dimension: ascending thin-layer homochromatography on plates of DEAE-cellulose using homo-solvent B (refs 14, 15). *a*, T₁ fingerprint of the entire 9S mRNA transcript labelled with α -³²P-UTP and purified by polyacrylamide gel electrophoresis, as shown in Fig. 3. *b*, T₁ fingerprint of a 100-nucleotide-long RNA fragment isolated from the 3'-terminal region of the λ b2 9S RNA transcript by hybridisation. Gel-purified, 9S mRNA (as in *a*) was hybridised directly to separated r-strand DNA obtained from the deletion-substitution mutant λ imm434. The filter-bound hybrids were trimmed with T₁ RNase and eluted from the DNA. Details of these hybridisation procedures will be described elsewhere. The RNA fragments obtained represent that portion of the P_R initiated, ρ -terminated mRNA transcript which extends beyond the right-hand boundary of the *imm434* region of non-homology (see Fig. 1 and Fig. 2a and b). *c*, Two-dimensional fractionation of the 3'-terminal oligonucleotides isolated on DBAE-cellulose from the 9S mRNA transcript shown in *a*. All procedures were as previously described^{11,17}. *d*, T₁ fingerprint of an RNA transcript which extends beyond the t_{R1} ρ -dependent site of transcription termination. Total RNA, transcribed in the absence of ρ -factor and labelled with α -³²P-GTP, was first hybridised on filters to the *Hae*III-*Hinc*II DNA fragment shown in Fig. 1b. After trimming the hybrid with T₁ RNase, the RNA was eluted and subsequently hybridised to purified r-strand DNA from λ imm434, as in *b*. The details of this two-step procedure, as well as alternative procedures which gave essentially identical results, will be published elsewhere. The RNA fragments obtained represent that portion of the P_R -initiated transcript which extends beyond the *imm434* boundary and spans the Y region and part of the *cII* gene. Numbered oligonucleotides indicate characteristic T₁ products which occur in the transcript preceding the t_{R1} -dependent termination site (see Fig. 2b). Lettered oligonucleotides represent characteristic RNase T₁ products which occur distal to the t_{R1} site. (a) UUAUCUAAAG(G); (b) AAUACUUACAUAUG(G); (c) CAAACAAACG(C); (d) CUCUACG(A); (e) CUUACAAAAUCG(C); (f) CAAUG(C), AAUCG(A), AACUG(A). These sequences were predicted by the DNA sequence (Fig. 2a) and also determined independently by standard RNA sequencing procedures¹³⁻¹⁵.

Table 1 Comparison of transcription preceding and distal to t_{R1}

Template	Oligonucleotides* preceding termination site (mole ratio)†					Oligonucleotides* following termination site (mole ratio)†			
	3	2	7	4	10	a	b	c	e
a 5-min labelling									
λ + (-rho)	0.96	0.98	—	0.93	1.00	0.94	0.93	0.95	—
λ + (+rho)	—	0.95	—	1.01	0.94	0.16	0.21	0.19	0.20
λcin (-rho)	—	0.91	0.96	0.92	—	0.92	0.90	—	0.94
λcin (+rho)	0.91	0.97	—	0.96	—	0.07	0.09	0.05	0.05
λcnc (+rho)	—	0.94	0.94	0.92	—	0.89	0.87	0.90	0.85
b Pulse labelling (-rho)(s)									
λ + 25	0.91	0.95	0.91	0.80	0.65	0	0	0	0
40	0.98	1.01	0.95	0.90	—	0.05	0.04	0.05	0.02
55	—	1.00	1.10	1.05	0.85	0.19	0.15	0.20	0.17
300	0.96	0.98	—	0.93	1.00	0.94	0.93	0.95	—
λcnc 25	0.94	0.89	0.85	0.80	—	0.74	0.70	0.67	0.65
40	—	0.95	1.00	0.95	—	0.90	0.87	0.85	0.87
300	—	1.01	0.97	0.96	—	0.93	0.90	0.90	—

a, *In vitro* transcriptions were carried out using the indicated DNA template as previously described¹¹ except for the addition of rifampicin (to 10 $\mu\text{g ml}^{-1}$) and the time of reaction (5 min). The P_R -initiated rightward mRNA (labelled with α - ^{32}P -GTP) was isolated by hybridisation and fingerprinted as described in Fig. 4d. b. Identical to (a), except that aliquots were removed at the indicated times. Reactions were stopped by the addition of EDTA (to 50 mM) and phenol. Hybridisation and fingerprinting procedures were identical to those used in (a). A reaction aliquot was also analysed after 10 s of transcription and none of the designated oligonucleotides were detected. Note that genetic selection procedures warrant that *cnc* mutants are always obtained in conjunction with the *cin* mutation (that is, $\lambda cin cnc$; see text, legend to Fig. 3, and ref. 30).

*Oligonucleotide numbers and letters are those designated in Fig. 4 and Fig. 2b. The *cin* mutation changes the nucleotide sequence of oligo no. 4; however, this alteration only slightly affects the position of this oligo on the two-dimensional fingerprint. Thus, the altered oligo can be readily monitored.

†Oligonucleotides were removed from the TLC plate by scraping and the cellulose powder was counted directly. Counts were then normalised relative to the number of labelled phosphates (that is, 1 or 2) which were incorporated into each oligo. Counts from oligonucleotides preceding the termination site (nos 3, 2, 7, and 4) were averaged and the mole ratios of all oligos determined relative to this average. Mole ratios are indicated only when averages were obtained from at least two independent experiments.

transcription preceding and distal to t_{R1} . The results (Table 1) indicate that in the presence of ρ , termination at t_{R1} is approximately 80% effective; 20% of the polymerases transcribe through t_{R1} . In the absence of ρ , no termination is detected.

These same procedures were also applied to the isolation and analysis of the P_R -initiated early mRNA species obtained from λ -infected cells pulse-labelled with ^{32}P - H_3PO_4 in various different conditions and at varying times after infection (our unpublished work). The results indicate that *in vivo*, transcription termination of the early *cro* mRNA occurs at the same site and over the same four or five residues as *in vitro*. In addition, termination at t_{R1} *in vivo* is completely ρ -factor-dependent and efficient read-through at the site requires expression of the λ *N* function. In the presence of both *N* and ρ , *N* (that is, anti-termination) predominates and in a wild-type infection of normal host cells, termination at t_{R1} can only be detected early after infection, before *N* expression. In the absence of *N* function, however, (that is, infection in the presence of chloramphenicol or with an *N* mutant) termination is 50–70% effective at t_{R1} , somewhat less than was found *in vitro* with ρ present. If a host defective in ρ is used (as, for example, ρ_{1515}), no termination at t_{R1} is detected. Thus, results similar to and consistent with those documented here for *in vitro* transcription have also been obtained *in vivo* (our unpublished work).

Structure at t_{R1}

Certain common structural features have been noted at the 3' termini of RNA transcripts obtained from a variety of different systems and are thought to result *in vivo* and/or *in vitro* from transcription termination^{20–24}. These include: (1) a run of consecutive uridylylate residues at the 3' end of the RNA transcript; (2) a G·C-rich region of variable length (4–12 consecutive residues) immediately preceding the uridylylates; and (3) a potential RNA 'stem and loop' structure near the end of the transcript (reflecting a region of hyphenated dyad symmetry within the DNA template). For those RNAs which can be prepared *in vitro*, the RNA polymerase seems to recognise the proper signal and terminates transcription at the appropriate site on the DNA, in the absence of any additional factors (that is, independent transcription termination). The termination

factor ρ seems to help effect termination at certain of these sites both *in vitro*^{11,25} and *in vivo*^{26,27}.

Transcription termination at the t_{R1} site clearly differs both structurally and functionally from those sites already described. Termination both *in vivo* and *in vitro* is completely dependent on ρ . The transcript which results does not end in a run of uridylylates, although a U-rich region does occur 13–17 residues before the actual point of termination (Fig. 2a and b). There is also no apparent G·C-rich region preceding the site. Although these two structural features may be essential in the independent termination reaction, they are not required for the ρ -dependent event at t_{R1} . The most striking similarity between the t_{R1} site and other sites of transcription termination is the occurrence of the hyphenated dyad symmetry in the DNA (Fig. 2a) preceding the termination site. The importance of this feature is discussed below.

A second ρ -dependent site of transcription termination has been defined *in vitro* beyond the end of the *E. coli* tyrosine tRNA gene²⁸. As observed for t_{R1} , termination is heterogeneous within the site and no uridylylate-rich sequence is found at the 3' end of the transcript. The most striking similarity between this site and t_{R1} , however, is the occurrence of a common sequence, CAATCAA, near the point of termination. The importance, if any, of this sequence to the ρ -mediated termination event is unclear. The termination region of the tRNA gene differs in several ways from other known sites of transcription termination. For example, no potential stem and loop structure immediately precedes the termination site and an extensive G·C-rich region just follows the site. The significance of these and other features of this terminator region are discussed in ref. 28.

Mutations affecting termination

Various mutations which affect the ability of λ to grow either lytically or lysogenically map in the Y region (*cin*, *cnc*, *cY*, *cI7*, IS elements)^{29–34}. One of these, *cin*, is a *cis*-acting mutation which increases the ability of λ to lysogenise its host, even in the presence of certain other mutations which alone permit little or no lysogeny (for example, *cII*, *cIII*, *cY*)^{29,30}. A secondary set of mutations, *cnc*, map just to the right of *cin* and effectively

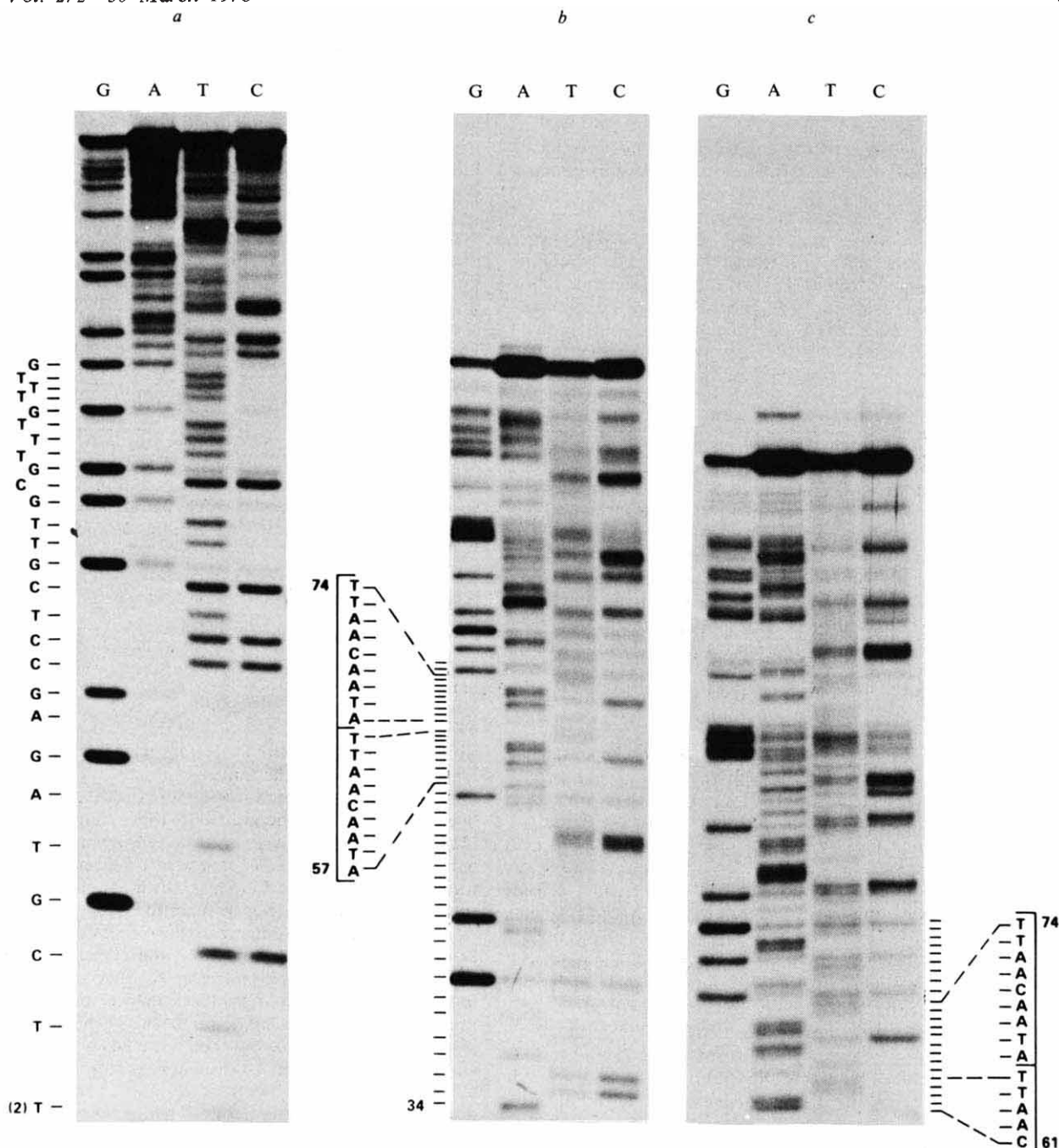


Fig. 5 Representative autoradiographs of DNA sequencing gels displaying the results of the chemical sequencing methods¹⁶ as applied to the λ DNA fragment extending to the left of the *Hinf* site shown in Fig. 1b. The *Hae*III-*Hinc*II fragment (Fig. 1b) was cut with *Hinf*, dephosphorylated with bacterial alkaline phosphatase, and 5' end labelled with γ -³²P-ATP using T4 polynucleotide kinase⁵⁷. The resulting labelled fragments were digested with *Ava*I and separated by electrophoresis on a 5% polyacrylamide gel. The *Ava*I-*Hinf* fragment was well resolved and could be eluted from the gel in pure form. This fragment was sequenced directly. (a), *Ava*I-*Hinf* fragment obtained from wild-type λ DNA; first discernible residue is a T, two nucleotides from the labelled end (position 374, Fig. 2a). (b), *Ava*I-*Hinf* fragment obtained from λ c17; first discernible residue is an A, 34 nucleotides from the end. Note the sequence duplication (9bp) at positions 57-65 and 66-74 (residues 328-320 and 319-311, respectively, Fig. 2c). (c), Identical to (b) except for longer electrophoresis such that first discernible residue is 61 nucleotides from the end (position 324, Fig. 2c), within the duplicated region.

negate the *cin* phenotype, thereby shifting phage development back toward conditions favouring lysis³⁰. The proximity of these mutations and their opposing effects on development suggest that the region defined by these and other Y region mutants encodes information which is critical to the regulation of lytic and lysogenic modes of growth in λ .

We have determined the nucleic acid sequence changes of the *cin* and *cnc* mutations (Fig. 2a and b) and have examined their effects on rightward transcriptions in the region surrounding the *t*_{R1} site. Hybridisation and fingerprint analyses were again used to monitor transcription in both the *in vitro* and *in*

vivo systems. The *cin* mutation occurs 30 residues 5' to the point of termination and increases p-dependent transcription termination at *t*_{R1}. We compared the relative efficiencies of transcription termination at *t*_{R1} using λ + and λ *cin* DNA templates (Table 1). In contrast to the 80% termination efficiency observed for the wild-type template *in vitro*, the *cin* DNA exhibited >90% termination efficiency in the presence of p; less than 10% of the transcripts were extended beyond *t*_{R1}. *In vivo*, the effect of *cin* was even more pronounced (our unpublished work). Termination at *t*_{R1} is normally detectable only at early times after λ infection (that is, before *N* expression).

In contrast, the *cin* mutant terminates transcription at t_{R1} with relatively high efficiency ($\sim 60\%$), even after *N* expression. If *N* is mutated (for example, $\lambda NN^- cin$), only 10% of the polymerases that reach t_{R1} are able to transcribe through; 90% of rightward transcription is terminated. These results are similar to those obtained *in vitro*. By using a ρ -defective host, we have shown (unpublished) that all termination at the site remains dependent on ρ factor.

The *cin* mutation has at least two structural effects at the t_{R1} site. First, the single base change extends the region of hyphenated, dyad symmetry within the DNA and thus the potential length and stability of the RNA (or DNA) stem and loop structure which can form near the t_{R1} site (Fig. 2b). The increase in stability of the stem region contributed by the additional A·U base pair is relatively small. If, however, this region of the RNA or DNA were involved in multiple interactions (see below, *cY* mutants), then relatively small changes in stability might result in structural equilibrium shifts large enough to have major functional effects. In this case, at least one such effect would be an increase in rightward termination efficiency at the t_{R1} site. Second, a 'heptamer' sequence, TATGGTG (positions 277–283, Fig. 2a), similar to those involved in the formation of stable RNA polymerase preinitiation complexes at sites of transcriptional initiation³⁵, occurs 27–33 residues upstream of the termination site. The *cin* mutation also alters the central purine position of this sequence, G→A (Fig. 2a). If this heptamer sequence is important to the termination event, then the mutation may affect (by stabilising, for example) the interaction of RNA polymerase with this site in such a way as to increase ρ -dependent transcription termination at t_{R1} .

Three independently isolated *cnc* mutations have been examined. The sequence changes of two of these are shown in Fig. 2a and b; the third has a different, but as yet undetermined, base change and occurs between positions 284 and 287 in the sequence. All of the *cnc* mutations interfere with ρ -dependent transcription termination at t_{R1} . *In vitro*, in the presence or absence of ρ , little, if any termination is detected at the *cnc* altered t_{R1} site (Fig. 3b and Table 1). *In vivo*, similar results have been obtained (unpublished) both in the presence and absence of *N* function. The *cnc* mutations are located 4 to 6 base pairs to the right of *cin* (Fig. 2a and b) adjacent to, not within, the 'heptamer' sequence. Most strikingly, these base changes all reduce the dyad symmetry of the DNA in this region and thus correspondingly reduce the stability of the potential stem and loop structure which can form within the termination site. The absence of ρ -dependent termination in *cnc* mutants may result from their interference with this structure.

Thus, the phenotypically opposing *cin* and *cnc* mutations are alterations in the t_{R1} termination region which have opposite effects on termination function, and correspondingly opposite effects on the stability of the potential secondary structure within this region. Comparison of the various stem and loop structures occurring at all the known transcription termination sites indicate no obvious primary sequence homologies. The independent termination sites (sites at which RNA polymerase stops independently of added factors), however, have base-paired stem structures of greater stability (that is, more potential base pairing and/or more G·C pairs) than the ρ -dependent t_{R1} site. Perhaps differences in the overall efficiency and factor dependence of the termination sites reflect, in part, the stability of this structural feature.

RNA polymerase pause site

Termination of transcription is thought to consist of a number of discrete steps. Before release of the RNA transcript and dissociation of the RNA polymerase from the DNA, the polymerase presumably stops, or pauses, at some specific signal encoded in the DNA template. Gilbert³⁶ has suggested that a G·C-rich region found 10 base pairs (one turn of the helix) upstream from a termination site may impede the progress of the RNA polymerase. We have examined, in kinetically con-

trolled *in vitro* transcription reactions, the ability of RNA polymerase (initiated at the P_R promoter) to move through the region surrounding t_{R1} in the absence of ρ factor. Aliquots were withdrawn from an *in vitro* transcription reaction (carried out in the presence of rifampicin at 37 °C with a relatively high triphosphate concentration, $\geq 100 \mu\text{M}$) at 15-s intervals after initiation of transcription. The RNA was isolated, fingerprinted, and the relative intensities (molar ratios) of the appropriate oligonucleotides proximal and distal to t_{R1} monitored (as described above). The results (Table 1 and M. R. and D. W., unpublished) indicate that even in the absence of ρ factor, RNA polymerase undergoes a substantial kinetic 'pause' within the t_{R1} site, before it transcribes the distal *cII* region. The signal specifying the pause at t_{R1} is presumably encoded in the template and does not seem to require a region of extensive G·C base pairs.

Similar experiments were carried out with the *cnc* mutations, which eliminate ρ -dependent termination at t_{R1} . Little, if any, pause in transcription was detected with these mutants (Table 1 and M. R. and D. W., unpublished). The results suggest that the pause may be a necessary step in the termination event. Because, in the absence of ρ , the pause at t_{R1} is only transient, in contrast to other sites of transcription termination which have been studied, one major functional difference between various termination signals may be the overall efficiency with which the RNA polymerase can be slowed or stopped at these sites.

N Recognition site (*nutR*)

The *N* product of λ allows extension of transcription from both the P_L and P_R promoters beyond their respective termination signals^{5,6}. The *N* product of phage λ will not function in the transcription of phage 21 nor will the *N* analogue of phage 21 work with λ (ref. 37). In contrast, the heteroimmune phage $\lambda imm434$ and λ have the same functional *N* gene. This suggests that *N* may recognise a region in the genome between the boundaries of *imm434* and *imm21* (Fig. 1). Indeed, for leftward transcription from P_L , a site of *N* recognition has been genetically defined to the left of *imm434*, between the startpoint of leftward transcription, S_L , and the gene *N* (ref. 38). Mutations at this site (*nutL*) are defective in recognition of the *N* gene product and thereby prevent antitermination of leftward transcription at various terminator regions within the left arm of the λ genome.

For rightward transcription from P_R , our results (unpublished) indicate that *N* acts to prevent termination at t_{R1} . This suggests a possible recognition signal for *N* upstream (5') from the t_{R1} site of action but to the right of the *imm434* boundary (Figs 1 and 2). Comparison of the nucleic acid sequence of this region with that determined by Blattner and Dahlberg³⁹ for the 5'-terminal region of the P_L -initiated transcript indicates the occurrence in each region of almost identical sequences (16 of 17 consecutive nucleotides) appropriately positioned in the same orientation relative to the direction of transcription. (*nutR* is shown in Fig. 2a, *nutL* differs only by an $\begin{smallmatrix} A & G \\ T \rightarrow & C \end{smallmatrix}$ change at the position equivalent to 252.) The sequence displays a hyphenated, twofold rotational symmetry that gives rise to a potential stem and loop structure in the RNA transcript of these regions (Fig. 2b).

We have recently determined the sequence changes that occur in three independently isolated $\lambda nutL$ mutants. These mutations were found to occur at two different sites within the 17-nucleotide-long sequence (our unpublished work with J. Salstrom). These data strongly suggest that these two sequences are in some way involved in *N* recognition before it acts at downstream terminators. It is not yet known whether recognition involves structure in the DNA or RNA.

Terminator and promoter— $\lambda c17$

The *c17* mutation of bacteriophage λ lies in the intercistronic region between the *cro* and *cII* genes³². This mutation is thought to be a small DNA insertion⁴⁰ which creates a new promoter site for rightward transcription by RNA polymerase from

within the Y region of λ . Although *cII* function (necessary for repressor establishment) is expressed in the mutant⁴¹, the phage cannot lysogenise its host, presumably because of constitutive expression of the DNA replication functions O and P (ref. 42).

We have determined the nucleic acid sequence and examined *in vitro* RNA transcription in the region surrounding the *cI7* mutation (Fig. 2c). Structural analysis indicates that the mutation results from an exact DNA duplication of nine consecutive base pairs occurring within and adjacent to the t_{R1} site (positions 311–319 of the wild-type sequence). This small duplication, which may well result from a DNA replication error rather than by a DNA insertion mutation, extends an A·T-rich segment of the DNA (eight of the nine residues are A·T) and creates a 'heptamer' sequence³⁵, (TATAATT) at the junction of the repeat (Fig. 2c). Our transcription studies indicate that *in vitro*, RNA polymerase binds efficiently at this site and initiates transcription six base pairs to the right of the heptamer at residue position 329 (Fig. 2c)⁶². Thus, the *cI7*-initiated transcript atypically contains a template-specified 5'-cytidine triphosphate (CTP). Although potential purine startpoints occur at residue positions 327 and 331 (four and eight base pairs respectively from the heptamer), little if any initiation occurs from these positions. This suggests that at the *cI7* promoter the steric constraints on promoter function outweigh the necessity for RNA polymerase to initiate transcription with a purine triphosphate. In our transcription conditions¹¹ the *cI7* promoter is somewhat weaker (three- to fourfold) than the P_R promoter. This was also suggested from *in vivo* studies^{43,63}. Perhaps the relative inefficiency of the *cI7* promoter is a direct result of the pyrimidine triphosphate initiation requirement. The *cI7* duplication and its promoter activity do not seem to disrupt the function of the ρ -dependent t_{R1} terminator site. Transcription initiated normally from the P_R promoter of $\lambda cI7$ is still sensitive to ρ termination at the appropriate t_{R1} site *in vitro*. It is not known, however, whether polymerase entry and initiation at the *cI7* promoter affect proper transcriptional regulation at the t_{R1} site *in vivo*.

Although the heptamer sequence created by the duplication probably accounts for formation of the tight binding site for RNA polymerase, the nine base-pair duplication does not encode all the information sufficient for specifying promoter function. DNA structure 35–40 base pairs upstream from RNA polymerase initiation sites has been implicated in proper polymerase recognition^{36,44,45}. This suggests that part of the information required for polymerase recognition and entry at the *cI7* promoter must be derived from the DNA region surrounding the *cI7* mutation. We suggest that the t_{R1} terminator region, appropriately positioned 20–50 residues 5' to the *cI7* initiation site, may also be serving as a recognition signal for those RNA polymerase molecules which initiate transcription at the *cI7* promoter. This implies certain common features, both structural and functional, between the ρ -dependent transcription termination site and RNA polymerase entry and initiation sites. The possible existence of overlapping promoter and terminator signals in bacteriophage $\Phi X174$ has also been noted⁴⁶.

The startpoint of transcription from the *cI7* promoter is just upstream from the presumptive ribosome binding site of the indicated *cII* protein (Fig. 2). *In vivo*, the transcript initiated from the *cI7* promoter (as well as the P_R -initiated polycistronic mRNA) is apparently translated into *cII* protein⁴¹. *In vitro* translation of the *cI7*-initiated mRNA transcript supports this contention and further indicates that two proteins are translated from the mRNA encoding the *cII* region (B. Paterson and M. R., unpublished). These two polypeptides share the same translational reading frame and have identical carboxy termini. They differ only at their amino-terminal ends, the larger protein having 20–30 additional amino acids at its N terminus. Ribosome binding experiments (B. Paterson and M. R., unpublished) indicate that the larger protein starts at the AUG triplet at position 338 in the sequence (*cII*, Fig. 2a). It is not yet known where the second, smaller polypeptide begins. No evidence yet exists for the occurrence of two *cII* proteins *in vivo*.

Insertion element—IS2

The phage $\lambda r32$ contains an IS2-type insertion element in the Y region^{34,47}. This ~1,200 base-pair DNA segment occurs as a normal component in the bacterial chromosome as well as in extrachromosomal plasmids, and seems to be involved in the transposition of genetic information within the bacterial cell⁴⁸. When inserted into a gene, the IS2 acts as a mutation and may exert polar effects on the expression of distally located genes⁴⁹. In $\lambda r32$ the IS insertion interferes directly with the expression of *cII* function and also exerts a polar effect (in the absence of N function) on the expression of the adjacent genes, O and P.

We have identified the exact site of insertion of the IS2 element in $\lambda r32$ and have determined the DNA structure at each end of the insertion element (Fig. 2d). The sequence is consistent with the contention that the IS2 DNA segment inserts between two adjacent base pairs (that is, within a single internucleotide-linkage) without deleting any of the original DNA structure. There is a direct sequence repeat (GAAAT) at each end of the insertion (Fig. 2d), however, and since this sequence occurs only once in the phage DNA, the repeat must either come from the IS2 element itself, or result from a duplication of structure at the time of the insertion event. Similarly, sequence repeats have been found at sites of IS1 insertion^{55,66}. The duplicated nucleotide sequences which occur at the various sites of both IS1 and IS2 insertion are different^{50,55,66}.

Although the phage $\lambda r32$ is defective in *cII* function³⁴, the site of IS2 insertion is not in the structural region of the *cII* gene. The insertion does occur, however, within the presumptive ribosome-binding region of *cII* and thus presumably interferes with ribosome recognition and translation initiation of the *cII* gene. A second IS2 insertion has been defined near the startpoint of transcription of the *gal* operon (R. Musso, personal communication). This insertion interferes with expression of the *gal* epimerase (*E*) gene without affecting the structural coding region of *E*. Apparently, the IS again has affected proper ribosome recognition. A preliminary comparison of the sequences surrounding these two sites of IS2 insertion has been published⁵⁰.

Control of repressor establishment—*cY* region

λcY mutants, which lie within the Y region, interfere with repressor synthesis during the establishment of lysogeny (ref. 31 and our unpublished work). These closely linked, *cis*-acting mutations seem to define a site of regulation, the function of which is required for repressor expression. One suggestion is that the *cY* region is a promoter site (*pre*, Fig. 1) for leftward transcription into the *cI* gene^{51–53}. Alternatively, it has been suggested that the *pre* promoter for *cI* transcription is, in fact, the promoter for 'oop' RNA transcription, *Po*, which lies to the right of the *cII* gene⁵⁴ (see Fig. 1). If this is the case, then the *cY* region would in some way regulate the elongation of this mRNA transcript initiated several hundred base pairs upstream (perhaps by way of an attenuator-type mechanism). The primary structure of this region alone does not allow us to discriminate between these two possibilities.

Genetically, a fine structure map of the Y region produces two distinct but tightly linked clusters of *cY* mutations (our unpublished work). We have determined the sequence changes associated with four independently obtained *cY* mutants (Fig. 2a), two from the left cluster (*cY1*) and two from the right (*cYr*). The structural analysis confirms the relative genetic locations of these mutations and indicates that each mutation results from a single base pair alteration.

Structurally, the *cY* mutants also seem to define two adjacent, but separate sites (Fig. 2a). The *cY1* mutations are located to the left of the indicated *cII* structural gene, within a short imperfect inverted repeat sequence (positions 331 to 338 are rotationally symmetric with positions 317 to 324, Fig. 2a) which occurs just to the right of t_{R1} . These mutations occur within the presumptive ribosome-binding region of the *cII* protein; three and six residues, respectively, preceding the *cII* AUG initiation codon; however, these mutants seem normal in *cII* function.

The *cYr* mutations are located within the coding region of the indicated *cII* protein. Each of these mutations, however, results in a third (wobble) position codon change which does not alter the amino acid sequence of the *cII* protein. Genetic experiments (unpublished) suggest that the region defined by the *cYr* mutations may, in fact, be the site of action of the *cII* protein. Thus *cII* may act at a site which concomitantly encodes its own structural information. The most striking feature of the region defined by the *cYr* mutations is again one of structural symmetry. The region encompasses a 14 basepair nucleotide sequence (residues 346–359, Fig. 2a) which is repeated in 12 of 14 consecutive positions in the opposite orientation within the *t_{R1}* site (residues 286–299). Possible implications of this feature will be dealt with below.

The mechanism(s) by which the *cY* mutations interfere with repressor synthesis is as yet unclear. The nature of the *cY* base changes and their relative positions do not seem to be analogous to classical promoter-type mutations (for review see ref. 36). If the *cY* region does promote leftward transcription of repressor, then this promoter and its regulation must be different from those previously examined. A more extensive genetic and structural analysis of mutations affecting *cY* function will be described elsewhere.

Potential interactions and overlaps

We have defined a set of regulatory elements positioned within the *Y* region of phage λ which regulate transcriptional and translational expression of this region. These elements seem to function in the control of both the lytic and lysogenic modes of viral development. The structure and relative positioning of these sites suggests that regulation of this region is a function of the molecular overlaps and structural interactions which may occur between the various control sequences.

In the previous section, a striking symmetry feature (namely, inverted repeat sequences) was noted between the *cY* region (controlling transcriptional expression leftward) and the *t_{R1}* termination site (controlling early transcription rightward). The length and relative positions of these inverted repeat sequences suggest the possibility of some common control mechanism which links the two opposing regulatory sites functionally. For example, the potential exists for a direct interaction of the sites resulting from the formation of base-paired secondary structure which might occur either in the DNA template or in the RNA transcripts of this region. Alternatively, the similarity in sequence of the two sites may denote two separate, but related recognition sites of a common control protein (for example *cII*). This protein could, in turn, mediate (either promote or interfere with) structural interactions between the two sites. We suggest that some interaction between *t_{R1}* and *cY* (in combination with a variety of viral- and host-encoded factors) couples the regulation of bidirectional transcription within this region.

We have also described another inverted repeat sequence which occurs within the *t_{R1}* termination site and which was thought to be important in the control of terminator function. The sequences involved in the formation of this 'termination structure' overlap with that implicated in the *cY* interaction. Thus, the additional possibility exists that the *t_{R1}*–*cY* region may exhibit alternative structural conformations, each exclusive of the other. Furthermore, it would be the equilibrium between the multiple conformations and the factors affecting this equilibrium which would regulate the bidirectional transcription through this region. A somewhat analogous form of transcriptional control based on alternative potential secondary structure has been postulated for attenuator control of the tryptophan operon⁶⁴.

Other possible structural interactions and overlaps occur within the *Y* region. The possibility of overlapping promoter and terminator sites in the phage λ c17 has already been discussed. In addition, the proximity of the recognition site for *N* function (*NutR*) and the *t_{R1}* termination site allows for

the possibility of some direct interaction between *N* protein and RNA polymerase while each is situated at its respective site. A third example involves the overlap between the *cY* region and the presumptive site for ribosome recognition and initiation of translation of *cII* protein. Although overlapping functions may simply reflect an evolutionary mechanism for conservation of genetic coding potential, these overlaps may also have important regulatory significance. For example, a model has been suggested whereby *cII* function acts within the *cY* region to increase leftward transcription while concomitantly regulating its own expression (ref. 53 and our unpublished work). What certainly becomes evident is the number and complexity of potential molecular interactions which may occur within the λ *Y* region. These interactions and their temporal control ultimately lead to the delicate balance achieved by λ in expressing both lytic and lysogenic patterns of development in a single viral infection.

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A ρ -dependent termination site in the gene coding for tyrosine tRNA su_3 of *Escherichia coli*

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A set of partially overlapping DNA restriction fragments that support promoter-dependent transcription of the tRNA^{Tyr} gene of Escherichia coli has been used to study site-specific termination in vitro. Transcription termination occurs at a specific site 224–226 nucleotides beyond the end of the structural gene and is completely dependent on ρ -factor. Certain features of this site suggest differences from other termination sites previously studied. A role for specific sequence recognition is suggested.

INITIATION and termination of RNA synthesis are two discrete steps in gene expression. These two events occur at different specific sites along the DNA template and are due to certain sequences or structural features which act as signals for the transcribing RNA polymerase. Both events can be modulated by the encoded sequences themselves or in conjunction with specific transcriptional control factors (see ref. 1 for review). In *Escherichia coli* the correct initiation of RNA synthesis is absolutely dependent on the presence of the σ factor². The ρ factor, on the other hand, causes termination of RNA synthesis *in vitro*³, and there is evidence that this termination factor is essential for *in vivo* function. However, until now, all termination sites studied *in vitro* have been independent of the ρ factor to varying degrees^{5–9}.

We report here an *in vitro* analysis of a completely ρ -dependent site-specific termination in the tyrosine tRNA su_3 gene (tRNA^{Tyr}). Our results suggest that in addition to the possible role of structural features in site-specific termination, specific sequences in the DNA template may also play a part in the ρ -polymerase termination reaction.

Extensive use has been made of a family of small DNA restriction fragments that support promoter-dependent transcription of the tRNA^{Tyr} gene and that extend for different distances in the downstream direction^{10,11}. Using this same *in vitro* system, we have previously shown that (1) purified RNA polymerase, in a σ -dependent reaction, initiates RNA synthesis *in vitro* with the same sequence as it does *in vivo*; (2) no termination of RNA synthesis occurs within the 70 bases following the CCA end of the tRNA sequence in the absence or presence of the ρ factor; and (3) the two RNA species coded by the phage $\Phi 80psu_{III}^+$ are transcribed as a common precursor.

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Effect of ρ on transcription from various DNA restriction fragments

The DNA fragments used in this study were derived by digestion of $\Phi 80psu_{III}^+$ DNA with various restriction endonucleases. The three major fragments, *Cla*, *HpaII* and *Bla*, extend 73, 372 and approximately 880 base pairs, respectively, beyond the sequences coding for the mature tRNA (see Fig. 1). The transcripts made by purified RNA polymerase from these DNA fragments and the effect of ρ factor on their lengths are shown in Fig. 2.

Transcription of *Cla* yields a main RNA species of 200 nucleotides, and the presence of ρ has no effect on the product size (Fig. 2, lanes 1 and 2). The number of nucleotides beyond those encoding the 3' end of the mature tRNA is known (see also Fig. 4), so this result indicates that transcription from the tRNA^{Tyr} promoter¹¹ proceeds to the end of this restriction fragment even in the presence of ρ .

Transcription of the *HpaII* fragment yields a major RNA species of 500 nucleotides which indicates that here also transcription proceeds up to (or close to) the end of the fragment. With this template, however, the presence of ρ has a strong effect on product size, and the resulting major RNA species has a length of 350 nucleotides (Fig. 2, lanes 3 and 4).

Transcription of *Bla* yields a more heterogeneous collection of RNA species. Although transcripts longer than 500 nucleotides are made, the majority of transcriptions do not proceed to the end of the fragment. The heterogeneity of the RNA pattern is due, in part, to contamination of the *Bla* fragment during isolation with other DNA fragments of similar size (see ref. 10). The ρ factor again reduces the size of the transcripts to yield a major RNA species of 350 nucleotides, the same size as is synthesised from the *HpaII* fragment in the presence of ρ (Fig. 2, lanes 5–8).

To make sure that the observed effect of ρ is not due to a nuclease contaminant in the preparation, transcripts made in the absence of ρ were incubated with the same amounts of ρ , and in the same conditions, as used in the transcription experiments. No change in the size or amount of transcript could be detected (data not shown). When different preparations of ρ were used, the same effect on RNA size was always observed (Fig. 2, lanes 6–8). The transcription products from *Cla*, *HpaII*, and *Bla* made in the absence or presence of ρ indeed contain the tRNA sequence because they can be processed by an *E. coli* S100 extract to yield the tRNA^{Tyr} (ref. 11, and unpublished results).

The patterns of lower molecular weight RNA species seen on the acrylamide gels are highly reproducible, although varying in

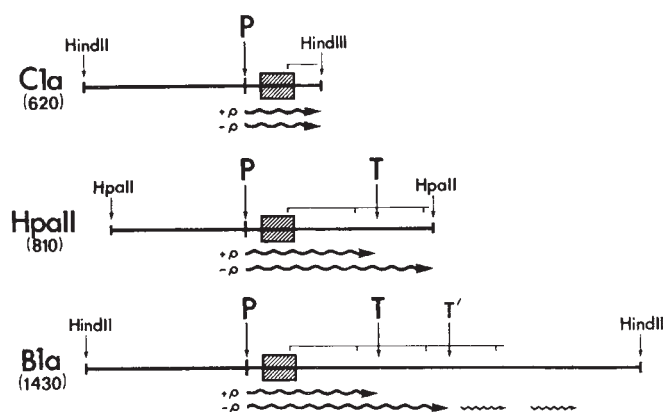


Fig. 1 DNA restriction fragments used as templates for promoter-dependent transcription of the tyrosine tRNA su_3 (tRNA^{Tyr}) gene. The isolation of *Cla* and *Bla* from the 'singlet' transducing phage, $\Phi 80psu_3^{+32}$ has been described previously¹⁰. The *HpaII* fragment is obtained by digestion of *Bla* with *HpaII* and purification by preparative polyacrylamide gel electrophoresis^{10,19}. The approximate size of the fragments in base pairs is given in parentheses^{10,19}. The 85 nucleotides coding for the mature tRNA sequence are indicated by the hatched box. These are preceded by 41 nucleotides coding for the 5' end of the primary transcript or precursor molecule³³. The end of the promoter and the startpoint of transcription is marked (P). The p-dependent termination site is marked (T), and a potential downstream termination site is marked (T'). The 178-base pair repeat unit (see Fig. 4) is indicated by overhead brackets. Promoter-dependent transcription (~~~~) in the presence and absence of p factor is indicated.

intensity (see also Fig. 3). These shorter transcripts may occur *in vitro* because of pausing of the RNA polymerase or because of inefficient termination events¹². The pattern might even be due to an alignment of polymerase molecules, as polymerase in these experiments is in 10- to 15-fold excess over DNA, and the distances between the bands observed are 50–60 nucleotides. The presence of p does not change this pattern, although it reproducibly decreases the amount of background synthesis especially of higher molecular weight bands (Fig. 2).

These results indicate that the *E. coli* tRNA^{Tyr} gene contains a relatively efficient p-dependent termination site *in vitro* approximately 225 nucleotides 'downstream' from the 3' end of the encoded tRNA sequence.

Influence of transcription conditions on p activity

The restriction fragment *Cla* provides a very useful means of separating the possible effects of p on initiation from those on termination; as *Cla* does not have a p-dependent termination region, the amount of tRNA^{Tyr} gene product is influenced solely by events at the promoter. Figure 3b shows that the effect of p factor on initiation is not dramatic, even over an approximate 10-fold range of polymerase to DNA (1.8:1 to 15:1). Although we see a reproducible small stimulation on addition of p factor (up to twofold, lanes 3 and 4, Fig. 3b legend), we could never achieve the very high stimulation reported for intact $\Phi 80psu_{III}^{+}$ DNA^{13,14}. Our results are therefore more in agreement with studies on p in other systems (refs. 3, 15, 16; see also discussion in ref. 17).

The catalytic action of p (ref. 15) at the termination site was established using the *HpaII* restriction fragment. Even a polymerase:DNA ratio as high as 24:1 (at a constant p:DNA ratio of 2:1) does not allow any additional polymerase molecules to read through the termination signal (data not shown). Addition of the polyanion heparin (20 μ g ml⁻¹) completely inhibits the action of p, either when the two are added simultaneously, or when p is preincubated along with the polymerase, DNA, and GTP + CTP (see ref. 11) before addition of the inhibitor (data not shown).

It has been observed that the activity of p is relatively

insensitive to ionic strengths up to 0.1 M KCl, whereas at high ionic concentration (>0.1 M KCl) p activity becomes gradually inhibited (see ref. 18 for review). In contrast, we find that termination at the tRNA p-dependent site is somewhat more sensitive to ionic concentration. Termination efficiency of the 350-nucleotide p-dependent product is not affected at 0.05 M KCl concentration; however, at 0.1 M KCl an approximate 50% inhibition of termination is observed (Fig. 3a). Note that the RNA chains that are extended beyond this p site at the higher salt concentration do not continue to the end of the fragment. Instead, a second major RNA product about 500 nucleotides in chain length is produced, suggesting the occurrence of a less sensitive, perhaps more efficient, termination site several hundred nucleotides downstream. This potential second termination site is discussed below in terms of the repeated DNA sequence that occurs within the tRNA gene.

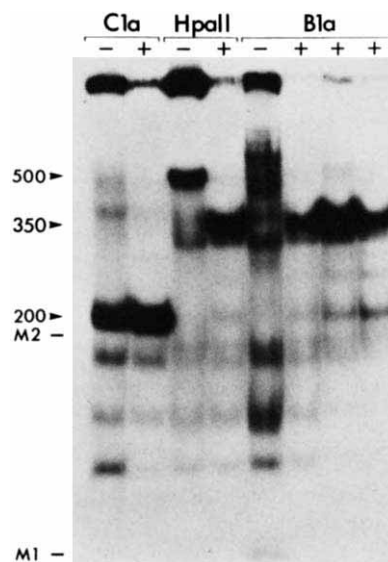


Fig. 2 Effect of p factor on transcription from various templates carrying the tRNA^{Tyr} gene. Standard conditions for RNA synthesis were 40 mM Tris-HCl pH 7.9, 10 mM MgCl₂, 10 mM 2-mercaptoethanol, 0.1 mM EDTA Na₃ and 15% glycerol in a final volume of 10 μ l. Nucleotides were added to a final concentration of 500 μ M CpC, 500 μ M GTP, 15 μ M each ATP, CTP and UTP. [α -³²P] UTP as the labelled triphosphate had a specific activity of 15,000 c.p.m. pmol⁻¹ (New England Nuclear). The dinucleoside monophosphate CpC was added to stimulate initiation of transcription¹¹. *E. coli* RNA polymerase (RNA nucleotidyltransferase EC 2.7.7.6.) was prepared according to Burgess³⁴ and was present at 1.5 pmol per 10 μ l. Termination factor p (prepared according to Roberts³) was present as indicated, and the DNA fragments were added as follows: lane 1, 0.15 pmol *Cla*; lane 2, 0.15 pmol *Cla* and 0.25 pmol p; lane 3, 0.06 pmol *HpaII*; lane 4, 0.06 pmol *HpaII* and 0.25 pmol p; lane 5, 0.08 pmol *Bla*; lane 6, 0.08 pmol *Bla* and 0.25 pmol p; lane 7, 0.08 pmol *Bla* and 0.2 pmol p (a gift from Dennis Kleid); lane 8, 0.08 pmol *Bla* and 0.2 pmol p (a gift from Elizabeth Bikoff). Incubation was for 60 min at 37 °C. RNA synthesis was stopped by the addition of 50 μ l electrophoresis loading buffer (7 M urea, 0.1% SDS, 90 mM Tris base, 90 mM boric acid, 4 mM EDTA Na₂, and the dye markers xylene cyanol and bromophenol blue), and heating for 2 min to 100 °C with subsequent quick cooling on ice. The RNA products were separated on a 4.5% polyacrylamide slab gel (20 $\times$ 20 $\times$ 0.2 cm) containing 7 M urea (acrylamide: N,N'-methylene bis acrylamide, 20:1). Electrophoresis was performed in TBE buffer (90 mM Tris base, 90 mM boric acid, 4 mM EDTA Na₂) at 50–70 mA until the bromophenol blue marker reached the bottom of the gel. M1 and M2 indicate the positions of the dye markers, bromophenol blue and xylene cyanol, respectively. The approximate size of the major transcription products (no. of nucleotides) is indicated in the left margin. The minor band which migrates just ahead of the p-dependent product (lanes 3–8) has not been analysed but is visually estimated to contain less than a few per cent of the total counts incorporated.

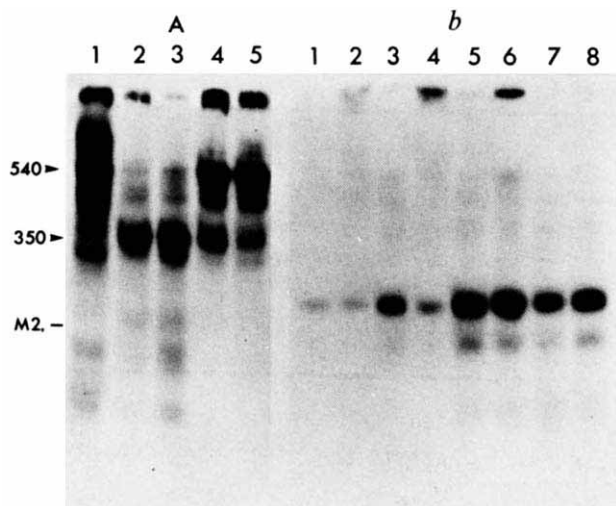


Fig. 3a, Effect of salt concentration on p -dependent termination. RNA polymerase (3.2 pmol) was preincubated with *Bla* (0.45 pmol) in the presence of CpC and GTP (500 mM each) in standard conditions (see Fig. 2) in a total volume of 35 μ l for 10 min at 37 °C. Aliquots of 7 μ l were withdrawn, and the remaining triphosphates were added to a final concentration of 15 μ M along with p (0.25 pmol per assay) and KCl as indicated. The mixtures (final volume 10 μ l) were further incubated for 50 min at 37 °C. This preincubation and the prior formation of initiation complexes are necessary because of the salt-sensitivity of the tRNA^{Tyr} promoter (see ref. 11). Transcription was stopped, and RNA products were analysed on a 4% polyacrylamide gel as described in Fig. 2. Lane 1, $-p$, $-KCl$; lane 2, $+p$, $-KCl$; lane 3, $+p$, 50 mM KCl; lane 4, $+p$, 100 mM KCl; lane 5, $+p$, 150 mM KCl. **b**, effect of p factor on promoter-dependent initiation of transcription at different polymerase:DNA ratios. Varying amounts of RNA polymerase were incubated in standard conditions with the *Cla* fragment (0.1 pmol per 10 μ l reaction) in the presence or absence of p factor (0.2 pmol per 10 μ l). Incubation was 45 min at 37 °C, and the RNA products were analysed on a 5% polyacrylamide gel (see Fig. 2). Incorporation of radioactivity into the main product was measured by Cerenkov counting of the excised gel band. The RNA polymerase:DNA ratios for each reaction and the amount of radioactivity in the excised band were as follows: lane 1, 1.8:1, $+p$ (1,388 c.p.m.); lane 2, 1.8:1, $-p$ (1,400 c.p.m.); lane 3, 3.7:1, $+p$ (3,841 c.p.m.); lane 4, 3.7:1, $-p$ (2,159 c.p.m.); lane 5, 7.5:1, $+p$ (7,611 c.p.m.); lane 6, 7.5:1, $-p$ (7,548 c.p.m.); lane 7, 15:1, $+p$ (5,692 c.p.m.); lane 8, 15:1, $-p$ (7,969 c.p.m.).

In addition to the triphosphate concentrations described in the legends to Figs 2 and 5, experiments have been carried out with several different combinations of three triphosphates at high concentration (0.5–1.0 mM each) and one at low concentration (10–15 μ M). The p -dependent termination of transcription was observed in all conditions (including an ATP concentration curve from 5 μ M to 1 mM), but the relative efficiencies in the various conditions were not quantified.

Experiments are in progress to further characterise the tRNA^{Tyr} gene termination reaction in a range of different conditions. In particular, this system affords the unique opportunity to investigate the effect of several important parameters (temperature, triphosphate concentration, dielectric constant of the solvent) on the relative termination efficiency at each of three successive potential termination sites (see below). These studies are being carried out in conjunction with an investigation of *in vivo* termination in this stable tRNA gene.

An unusual repeated structure in the distal portion of the tRNA^{Tyr} gene

Sequence analysis of the distal portion of the tRNA^{Tyr} gene and a discussion of the gene structure are presented elsewhere¹⁹. In this report we discuss those features that are specifically

related to termination of transcription. The most striking feature of the sequence is a 178-base pair unit that is repeated 3.14 times. The final sequence is presented in Fig. 4 in such a way as to facilitate a comparison of the repeated units. The strand of DNA which has the same sequence as the mature tRNA is shown. The first repeat begins within the sequence corresponding to the mature tyrosine tRNA, and a 19-base pair sequence from the 3' terminus of the mature tRNA occurs

Table 1 Quantitation of T1 oligonucleotides

Oligo no.	Sequence	Position in sequence	Mole ratio		
			<i>Cla</i> $-p$	$-p$	$+p$
2	CAAUU...G	164–178	0.94	0.94	0.96
3	CAAUC...G	342–356*	—	0.98	0.08
4	ACUCU...G	73–84	0.98	0.90	0.92
5	AACUU...G	146–157	0.95	0.95	0.94
6	AACUC...G	324–333	—	1.01	0.90
7	CAUUACCCG	32–40	1.00	0.97	0.92
10a	CUUCCCG	2–8	1.03	1.03	1.01
b	AAUCCG	{158–163 336–341}	1.03	2.05	2.03
c	ACUAAG	385–390	—	1.03	—
11a	ACUUCG	94–99	1.37	0.97	1.37
b	UCAUCG	88–93	1.37	0.97	1.37
c	AUAAG	9–13	1.37	0.97	1.37
13a	UUCCCG	49–54	0.71	1.02	1.02
b	UCCUG	{140–145 318–323}	1.43	2.03	2.05
14	CCCAUG	{179–184 357–362}	1.12	2.14	1.09
16	ACUCG	218–222	—	1.15	1.14
18	CUUCG	{202–206 380–384}	—	2.25	1.10

The promoter-dependent tRNA-containing transcript made from the *HpaII* fragment, either in the presence or absence of p , or from the *Cla* fragment in the absence of p , was isolated from a 5% urea gel as described in Fig. 5 legend. The ³²P label was either in GTP or UTP (in which case all ratios are corrected for the number of U residues in the oligonucleotide). The eluted RNA was digested with T1 ribonuclease and fractionated by electrophoresis at pH 3.5 in the first dimension and homochromatography in the second dimension^{35–37}. Each spot of radioactivity was quantified and then analysed by pancreatic RNase digestion. The pancreatic analysis, in conjunction with the mobility of the spot, permitted an unambiguous correlation of each T1 spot with those predicted from the DNA sequence (see Fig. 4). In the case of unresolved oligonucleotides in a spot (marked by brackets above), this analysis yielded the relative amount of each oligonucleotide in the spot. Those oligonucleotides which are different in the $+p$ and $-p$ reactions, or which are crucial in positioning the termination site are marked by an asterisk. Oligonucleotides not present in a particular transcript are marked by a dash.

without any alterations at the beginning of the four repeat units. There are only 14 sites at which one of the repeats differs from the others.

Also indicated in Fig. 4 are the 'downstream' endpoints of the restriction fragments used in the transcription studies reported here. As the size of the RNA product made from the *HpaII* or *Bla* fragments is dependent on the presence or absence of p factor, and the RNA product made from *Cla* is not, the p -dependent termination site must be between positions 199 and 497. The size of the p -dependent transcript from either *Bla* or *HpaII* further narrows the location of the termination point to the region of 320–380.

Precise location and sequence of the p -dependent termination site

Two different methods were used to determine precisely what portion of the DNA sequence specifies the p -dependent termination site. In the first, T1 oligonucleotides from the *HpaII* transcript made in the presence or absence of p were compared. These results are shown in Table 1 along with the T1

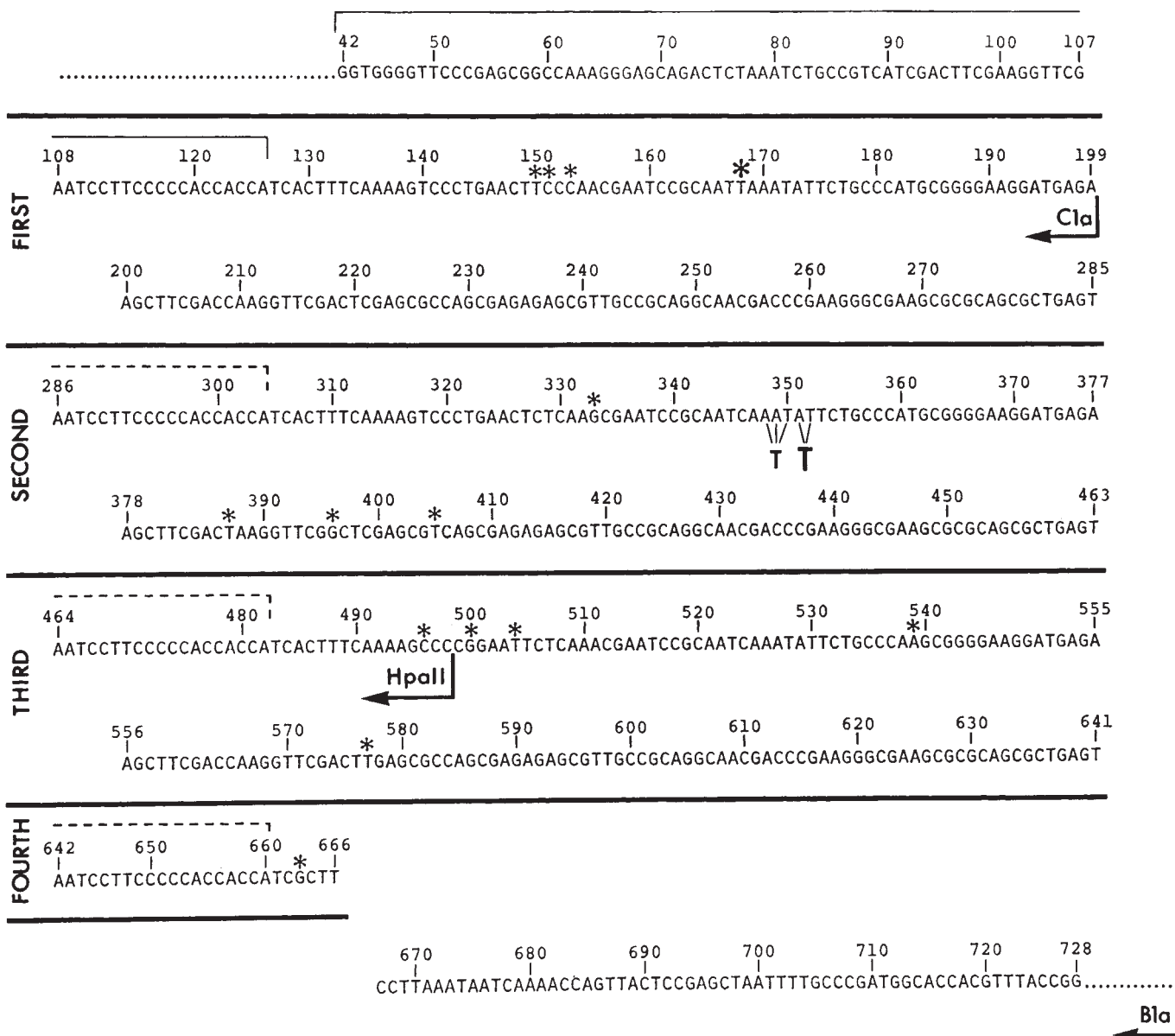


Fig. 4 Sequence of the distal region of the tRNA^{Tyr} gene¹⁹. The DNA strand having the same sequence as the tRNA is arranged to facilitate comparison of the 178-base pair repeating units. The first base shown (42) corresponds to the first base in the mature tRNA (the 41 nucleotides corresponding to the 5' portion of the primary transcript, or precursor^{11,33}, are not shown). The sequences corresponding to the mature tRNA are indicated by an overhead bracket, and that portion of this sequence which appears at the beginning of each repeat unit by a dashed bracket (see text and ref. 19). Positions in one repeat which differ from the others are marked by an asterisk. The location of the p-dependent termination of transcription is marked (T) (see text and Fig. 5). The downstream termini of the three restriction fragments used as templates for transcription in this study are also indicated (see Fig. 1).

oligonucleotides found in the transcript from *Cla*. Those oligonucleotides most crucial for placing the terminus of the +p transcript are marked with an asterisk. The absence, or reduction by one relative mole, of oligonucleotides 3, 10c, 14 and 18 in the +p transcript indicates that this RNA terminated before position 357 (the first base in oligonucleotide 14) but after position 341 (the last base in oligonucleotide 10b). This is confirmed by the presence in the +p transcript of oligonucleotide 10b as well as all of the oligonucleotides preceding this one in the sequence.

A second method was used to confirm the above results and to define more precisely the 3' terminus of the +p transcript. Highly labelled gel-purified transcript made from the *HpaII* restriction fragment in the presence of p was digested with T1 ribonuclease and passed over a column of dihydroxyboryl-substituted cellulose^{16,20}. All internal oligonucleotides, which

have a 3'-phosphate as a result of the T1 digestion, pass through the column; only those oligonucleotides derived from the 3' end of the transcript contain a cis-diol and are selectively retained.

The material eluted from the borate column was found to be heterogeneous (Fig. 5). Analysis of these oligonucleotides indicates that this heterogeneity is due to the sequential addition of bases within only one T1 oligonucleotide. The longest sequence obtained is CAAUCAAAU and could only be obtained from T1 oligonucleotide 3, as predicted from the experiments described above.

This result shows that termination occurs within a region of five bases, between positions 348 and 352. Position 351 seems to be the preferred termination point, where one third of all chains are terminated. Position 352 is the second strongest point of termination, and the remaining 30–35% of the

molecules have termini that are distributed about equally at positions 348, 349, and 350.

Features of the ρ -dependent termination of transcription

The transcription termination described here for tRNA^{Tyr}, and for the λ _{RI} site²¹, differs in several striking properties from other termination sites studied thus far. The latter all have transcripts which end in a stretch of four to seven uridine residues, and while they are enhanced by the ρ termination factor, they still show significant amounts of termination in its absence (see ref. 18 for review). In contrast to this, the termination described here is completely dependent on the presence of ρ factor, and the transcripts do not end with a stretch of U residues. An additional important distinguishing feature of these two ρ -dependent termination sites is their similarity in sequence, which is discussed below.

A common structural feature noted at other sites of transcription termination is the occurrence of a potential RNA stem and loop structure just before the end of the transcript (reflecting a region of hyphenated dyad symmetry in the DNA)^{5-9,21-23}. There is some evidence that suggests that this secondary structural feature is important to the termination reaction^{21,22,24}. The absence of such potential structures immediately upstream of the tRNA^{Tyr} termination site is therefore quite striking (see also ref. 19). [The two closest loci for potential stem and loop structures are 50 base pairs (positions 287-296 with 195-186) and 100 base pairs (positions 249-255 with 245-239) upstream from the tRNA^{Tyr} termination site (see Fig. 4)—considerably greater than the distance of 10-15 base pairs observed for other termination sites.]

A second feature of this site, which again contrasts with other sites of termination, is a relatively stable G-C-rich region (10 of 12 consecutive base pairs, positions 356-367) just beyond the A-T-rich termination site. Beyond other sites of transcription termination an A-T-rich region has been noted^{7,22,25}. In addition, a small cluster of four G-C base pairs occurs 10 base pairs upstream from the tRNA^{Tyr} termination site. Maizels and Gilbert^{12,26} have suggested that such a cluster of G-C residues will generate a pause in transcription 7-10 residues downstream. (The configuration of a high A-T region preceded by a G-C cluster of variable length is found in seven of the eight published prokaryotic termination sequences²⁷.)

Thus it is tempting to speculate that the apparent requirement for a 'structurally induced pause' in transcription may be satisfied at the tRNA^{Tyr} termination site by a stable helical region in the DNA template, both preceding and distal to the point of termination, which is difficult for RNA polymerase to read through. This further suggests that there may be a variety of alternative features encoded in the DNA template, various combinations of which may signal a transcription termination event.

An analogous situation, where a high G-C region affects the interaction of RNA polymerase with DNA, is seen in the promoter of the tRNA^{Tyr} gene¹¹. This analogy between the interaction of RNA polymerase at promoter and terminator sites can be extended further. Just as the termination of transcription is not a precise event, the initiation of RNA synthesis can also occur within a region of four or five base pairs if complementary dinucleoside monophosphates are provided^{11,28}, with certain positions within this variable region being preferred¹¹. RNA polymerase prefers purines to a high degree over pyrimidines as the initiating triphosphate. For termination U and A seem to be preferred as the terminal nucleotides. Although the homology among known promoter sequences is not great and is limited to small sections, severe changes in promoter function are brought about by the change of a single base¹. The results reported here indicate that the same situation can be true for the termination reaction as discussed in the following section.

Features of the termination in the tRNA^{Tyr} gene

In spite of the fact that the DNA sequence is reproduced in each of the 178-base pair repeat units with very few changes, termination of transcription takes place in the second repeat unit, at positions 350-353, but not in the first repeat unit (at positions 172-175) (see Fig. 4). Within a stretch of 100 nucleotides surrounding the termination region, there are only five positions that are different in the two repeat units (328, 329, 331, 333, 346; Fig. 4). One or more of these five bases may hold a key position in the sequence necessary for ρ -dependent

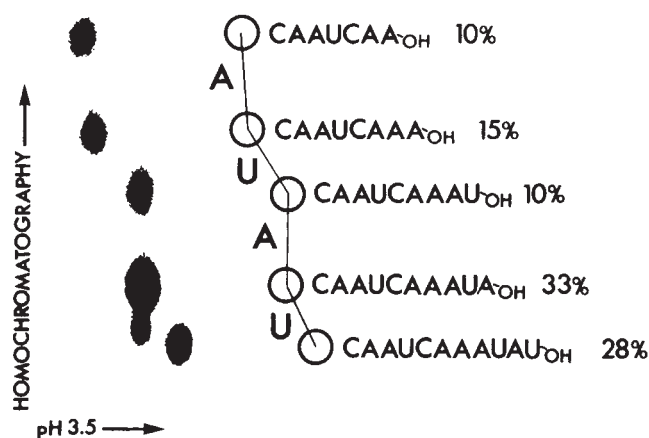


Fig. 5 Analysis of the 3'-terminal oligonucleotides of the promoter-dependent, ρ -dependent, transcript. The 360-nucleotide transcript from the *HpaII* reaction (see Fig. 2) was prepared and isolated as described in legend Fig. 2 with the following preparative modifications: total reaction volume, 30 μ l; *HpaII* restriction fragment, 0.3 pmol; RNA polymerase, 5.0 pmol; ρ factor, 0.6 pmol; [α -³²P]UTP at 15 μ M with a specific activity of $1-1.5 \times 10^5$ c.p.m. pmol⁻¹; GTP, CTP, and ATP each at 500 μ M. The radioactive band was excised from the gel and disrupted in a 5 ml syringe. The RNA was eluted with 1 M NaCl and recovered by ethanol precipitation as described previously¹¹. The RNA was digested with T1 ribonuclease and passed over a column of DEAE-cellulose²⁰ as described previously¹⁶. Oligonucleotides containing a 3'-terminal phosphate were eluted with solvent A (0.05 M morpholinium chloride, 0.1 M MgCl₂, 1.0 M NaCl, 20% dimethylsulphoxide, pH 8.7). Oligonucleotides which contain a nucleoside at their 3' end, and thus should be derived from the 3' terminus of the transcript, were subsequently eluted from the column by simple displacement with solvent A containing 0.1 M sorbitol. The 3'-oligonucleotides were desalted by dialysis against distilled water, concentrated under reduced pressure at 35 °C, and fractionated in the first dimension by electrophoresis on cellogel in 8 M urea at pH 3.5 and in the second dimension by ascending thin layer chromatography on plates of DEAE-cellulose (9:1, cellulose:DEAE-cellulose, 40 $\times$ 20 cm) using homochromatography solvent B³⁵⁻³⁷. The radioactive spots were eluted, quantified by Cerenkov counting, and subjected to further analysis by pancreatic ribonuclease digestion using standard techniques³⁵⁻³⁷. The schematic diagram next to the autoradiogram shows the sequence deduced for each spot, the single base differences deduced on the basis of mobility shifts between the spots, and the per cent of the total radioactivity found in each spot.

termination.

The base change at position 346 is the closest to the termination region and interrupts a stretch of 11 A-T base pairs. This difference seems to be the most likely candidate responsible for triggering the termination in the second repeat. The importance of this base change is further supported by the fact that it occurs within a seven-base pair sequence, CAATCAA, which is also found at the ρ -dependent λ _{RI} termination site²¹. These two facts taken together strongly suggest that this specific sequence may be recognised by the ρ factor or the ρ -polymerase complex.

It is interesting to note that the mutants of Maizels, which

affect RNA polymerase pausing, also occur 7–10 nucleotides upstream from the pause point^{12,26}—a spacing which approximates one turn of the DNA helix and which is also found in a critical region of *E. coli* promoters²⁹.

Inspection of the sequence in the third repeat suggests that it should also serve as a functional termination site (that is at positions 528–531), since it has the same sequence as the second repeat in the region of termination (Fig. 4). The experiments described here do not provide a rigorous test of this prediction since addition of ρ factor always results in termination in the preceding repeat unit. However, in those experiments where high salt was used to interfere with termination, we did find some suggestion of termination at the predicted site in the third repeat (Fig. 3a). The few base differences between the third repeat unit and the other two units create the potential for a stable seven-base pair stem and six-base loop structure (positions 499–519) which is quite similar to that observed at other termination sites as discussed above. If it is postulated that this stem and loop would serve to facilitate termination, then the three repeat units constitute a graduated set of termination sites of increasing strength. While there are several roles that might be ascribed to such an arrangement of termination sites, there is at present no *in vivo* evidence that bears on this question. The only *in vivo* tRNA_{I^{Tyr}} precursor isolated thus far extends just a few bases beyond the CCA terminus of the mature tRNA, and this is most likely the product of some processing enzyme rather than the primary transcript. The repeated structure of the tRNA_{I^{Tyr}} gene is not peculiar to the Φ 80psu_{III} transducing phage, but it does exist in the *E. coli* chromosome in all strains analysed so far (J. Rossi and A. L., in preparation).

It should be noted that, in addition to the sequence differences discussed above, the three potential termination sites are distinguished from each other by the structure of newly synthesised RNA behind the polymerase. For example, in the second and third repeats, the polymerase is linked to RNA that is an almost identical copy of the sequence being transcribed. Although at present there is no evidence, from this gene or others, that bears on this general question, the arrangement of restriction cut sites within the repeated units will facilitate the type of genetic rearrangements which might be useful in pursuing this point.

In addition to the experiments reported here using purified restriction fragments as templates, several other laboratories have been studying transcription of the tRNA_{I^{Tyr}} gene using intact Φ 80su_{III} transducing phage DNA as template. In most cases the sizes observed for the tRNA-containing transcripts were consistent with our results^{13,30,31}, although in one report¹⁴ a considerably smaller transcript would seem to correspond better to a termination in the first repeat unit.

One striking difference from our results is that in most of the experiments using intact Φ 80su_{III} transducing phage DNA as a template, the size of the tRNA-containing transcript was unaffected by the addition of ρ factor, although the quantity of tRNA sequences assayed was enhanced considerably. We do not have an explanation for this apparent termination in the absence of ρ factor, although it may be related to differences in RNA polymerase preparations. All ρ -independent termination sites analysed so far end with an uninterrupted sequence of four to seven U residues. Nowhere in the distal portion of the tRNA_{I^{Tyr}} gene, that is, within 500 nucleotides of the CCA terminus of the mature sequences, is there a sequence coding for an uninterrupted cluster of more than three U residues, or even a singly interrupted cluster of four or more U residues. In fact, the only moderately U-rich cluster within the repeated sequences comes from the termination site described in this report.

While it is possible that intact transducing phage DNA as a template does respond differently to ρ factor in termination of the tRNA_{I^{Tyr}} gene, there are some reservations. The problems of working with an extremely large multigenic

template are considerably more complex than with a purified restriction fragment in which initiation at the tRNA_{I^{Tyr}} promoter is readily monitored and the primary gene transcripts can be isolated with high purity.

Conclusions

In the system described above, the site-specific termination of transcription depends completely on the addition of ρ factor. This termination is sensitive both to salt and to heparin, and the action of ρ factor is catalytic. No striking stimulation of initiation of transcription by ρ factor (twofold or less) was observed.

If this ρ -dependent termination site also functions *in vivo*, then these results provide a solution to the long-standing problem of defining an unprocessed primary transcript from a tRNA gene and should greatly facilitate further progress in this area.

The ρ -dependent termination is not precise but takes place over a distance of approximately four or five adjacent base pairs, as has been observed for other termination sites not so tightly coupled to the presence of ρ factor. Whereas the immediate vicinity of the termination site is very high in A·T, the adjacent sequences on both sides are extremely rich in G·C.

In addition to the possible role of structural features in site-specific termination, our results suggest that specific sequences in the DNA template may in fact be recognised in the ρ -polymerase termination reaction.

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letters to nature

Coincidence of compact supernova remnants with three COS-B γ -ray sources

POSITIONAL coincidences and a 'near miss' of four small-diameter supernova remnants (SNR) with three of the 11 unidentified COS-B γ -ray sources¹ are reported here. I argue that it is unlikely that all four SNR- γ -ray source associations are accidental, and that their compactness probably indicates that their ages are not greater than about 10^4 yr. Although these four SNR are not associated with known pulsars, the radio emission²⁻⁴ in each case is centrally peaked which admits the possibility of a central pulsar. If these associations are physical the γ rays could originate from a remnant pulsar or could be due to cosmic ray interactions in the vicinity of the remnant⁵. The only two previously identified^{6,7} and confirmed⁸ γ -ray sources are the Crab pulsar and the Vela pulsar, both of which are associated with young ($\lesssim 2 \times 10^4$ yr) SNR. In these two cases the γ -ray emission is predominantly pulsed and therefore comes directly from the remnant short-period pulsars.

These coincidences were discovered in a comparison of the COS-B source list¹ with the SNR catalogue of Ilovaisky and Lequeux⁹. To estimate the chance probability of these associations we must specify in some detail the regions of the γ -ray search.

chance is only 0.5 and the probability of three or more is only about 1%. Therefore, it is unlikely that all three of these associations are accidental. The possibility of a physical relationship is strengthened because a fourth such SNR is positioned just outside one of the source error boxes.

These four SNR are listed in Table 1. $\Delta\theta$ is the angular separation of the SNR from the centre of the γ -ray error box. The diameters and distances are those of Ilovaisky and Lequeux⁹ calculated from the relationship of radio surface brightness and diameter determined by them. The 'age' of each remnant is calculated from the Sedov relationship¹² between the diameter, D , and the time, t , since the supernova: $D = 0.65 (E/n)^{1/5} t^{2/5}$ where E is the energy in 0.75×10^{51} erg and n is the interstellar atomic number density. This relationship is believed to hold in the early history of the remnant ($t \lesssim 2 \times 10^4$ yr) (ref. 11) until radiative losses become important. An empirical relation derived by Mills¹² differs only slightly from this if E/n is taken to be 1: in calculating the age we have assumed $E/n = 1$. This 'age' is a very crude estimate which may be incorrect to more than one order of magnitude. For example, application of this relation to the Vela and Crab pulsars gives ages which, for Vela, is reasonable (1.3×10^4 yr) but for the Crab is only 20 yr.

In calculating the chance probability of association of the

Table 1 Parameters of SNR candidates

γ -Ray Source	l''	SNR	Inside error box or		$\Delta\theta$	Diameter (pc)	Distance (kpc)	Age (yr)	$L\gamma$ (erg s $^{-1}$)
			b''	Near miss					
CG78+1	78.1 $^\circ$	1.8 $^\circ$	Inside		0.5 $^\circ$	9.4	4.0	0.8×10^3	2.7×10^{36}
CG327-0	{ 326.2 $^\circ$ 328.4 $^\circ$	-1.7 $^\circ$	Near miss		1.8 $^\circ$	11.9	4.2	1.4×10^3	1.0×10^{36}
		0.2 $^\circ$	Inside		1.1 $^\circ$	13.2	10.8	1.9×10^3	6.9×10^{36}
CG333+0	333.0 $^\circ$	0.8 $^\circ$	Inside		0.9 $^\circ$	12.5	16.6	1.6×10^3	3.3×10^{37}

The 11 unidentified γ -ray sources¹ (including one first seen by the SAS-2 satellite¹⁰) were compiled from a search limited to four galactic longitude ranges lying approximately along the galactic equator and spanning 160° in longitude. Two of the regions ($l'' = 54-94^\circ$ and $l'' = 302-342^\circ$) are generally toward the galactic centre and two ($l'' = 106-146^\circ$ and $l'' = 165-205^\circ$) are away. The SNR catalogue⁹ contains 112 entries (four of the original 116 having been deleted). Of these, 51 (excluding the Crab SNR) are confined to the region of the COS-B search and four of these are within a COS-B γ -ray source error box. The probability of four or more coincidences by chance is estimated to be about 25% based on the total size of the γ -ray error boxes (40 deg^2) and the approximate size of the SNR distributions ($40^\circ \times 2^\circ$ for each of the two regions toward the galactic centre and $40^\circ \times 8^\circ$ for the two away from the galactic centre). Therefore the number of coincidences is not conclusive evidence that any real association exists.

A different conclusion is reached, however, if only small-diameter SNR are selected. The evolutionary history of a 'typical' SNR expansion as given by Woltjer¹¹ indicates that a diameter of 22 pc is reached in about 2.2×10^4 yr. I have taken the smaller value of 15 pc as a reasonable selection criterion for SNR 'youthfulness'. When this selection is made only nine, again excluding the Crab, of the original 51 SNR remain and three of the nine are coincident with the γ -ray error boxes. The expected number of such coincidences on the basis of

two types of objects the distribution of SNR was assumed to be uniform within each of the four longitude ranges. In three of the four cases this is true; but in the region between $l'' = 74^\circ$ and 80° there is a clustering of seven remnants from the original list which may be related to the presence of a line-of-sight along the Orion arm of the galaxy. This clustering enhances somewhat the probability of an accidental association with the two COS-B γ -ray sources in this area. In this same region Cyg X-3 has been reported as a high energy γ -ray source by the SAS-2 group¹³. In a later observation, the COS-B group does not detect this source¹⁴, and, therefore, its variability, seen in the radio and X-ray regions, apparently persists to γ -ray energies.

In Table 1 the γ -ray luminosity (L_γ) is calculated in the interval 0.1-1 GeV assuming a mean photon energy of 250 MeV (ref. 15). Possible beaming effects which may be important have been ignored. Both the Crab and Vela γ -ray sources are nearly completely pulsed at energies above 100 MeV (refs 8, 16) and in many models, therefore, are beamed. If this pattern is followed for the SNR 'sources' then their true luminosity may be lower by a factor of 10 or more. Even so, the luminosities are large. For comparison the luminosity of the Crab above 100 MeV (assuming beaming into 1 sr) is about 0.5×10^{35} erg s⁻¹. In the case of CG327-0 we have ascribed the total observed γ -ray flux to the two candidates in turn. Of course, the source may be an unresolved 'double' as γ -ray detector angular resolutions are not yet sufficient to resolve such close pairs.

Radio maps²⁻⁴ of each of the four SNR reveal a centrally peaked pattern and in all cases except SNR 78.1+1.8 a spectral index in absolute value less than 0.25. As Chevalier¹⁷ noted, these characteristics may indicate the presence of a central pulsar. Although no radio pulsar is found coincident with these SNR, this may not be a strong argument against the detection of γ -ray emission from a remnant pulsar. If beam width is proportional to the fraction of the phase plot occupied by the pulse, then the γ -ray beam of the Crab is about 2.5 times its radio beam width and that of Vela five times larger. If this pattern persists, then a γ -ray emitting radio pulsar may very well be visible in γ rays at the Earth but not visible in the radio region, because of the increased size of the beam.

Future high resolution and high sensitivity radio and X-ray observations of these regions would be desirable to check various possibilities, for example, the presence of compact radio sources, hard X-ray sources, or fast pulsars within each remnant. Clark and Caswell¹⁸ have identified a smaller source within a larger angular area for SNR 326.2-1.7. A soft X-ray source, Cyg X-7, has been associated¹⁹ with SNR 78.1+1.8. Two recent investigations^{20,21} have listed faint X-ray sources found near the COS-B γ -ray positions. For the γ -ray sources listed here, X-ray sources are reported near CG078+1 (refs 20, 21) and CG327-0 (ref. 20). Two other sources away from the galactic centre (CG135+1 and CG189+1) have been tentatively identified with giant H(II) regions²².

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NGC1275: a BL Lacertae object?

NGC1275 APPEARS in Seyfert's list¹ of galaxies which have many high excitation emission lines in the nuclei. Seyfert remarks that all the emission lines in these galaxies are unusually broad. Khachikian and Weedman² separated the Seyfert galaxies into two classes. In class 1, the Balmer lines are broader than the forbidden lines; in class 2, the forbidden lines and Balmer lines are the same width. The lines in class 2 galaxies have full widths at half maximum intensity typically between 500 and 1,000 km s⁻¹. These widths are generally greater than those of the forbidden lines in class 1 objects, but the Balmer lines in class 1 objects are usually several thousands of km s⁻¹ wide. Class 2 Seyferts are also characterised by an extremely high [O(III)]:H β ratio. There are also significant differences in the UBV colour amongst Seyfert of class 1 and 2, the class 1 Seyfert's having larger UV excess. NGC1275 was called a Seyfert 2 galaxy by Khachikian and Weedman², as it showed no

indication of the broad, intense Balmer lines that characterised class 1 Seyferts, the forbidden lines widths and its [O(III)]:H β ratio were similar to those in class 2 Seyferts; however, the emission lines were very weak relative to the continuum. Moreover, there was an extended system of H α filaments having about the recession velocity of the galaxy³. It is not known how much of the emission lines seen in the nucleus comes from these filaments projected on it. I suggest here, on the basis of its weak emission line spectrum, its lack of the characteristic broad hydrogen lines and the variability and polarisation of its nucleus, that NGC1275 should be called a BL Lacertae object rather than a Seyfert galaxy.

The emission line spectrum of NGC1275 does not resemble that of any class 1 Seyfert. Adams and Weedman⁴ again claim that they could not detect any wings on H α and H β and that the forbidden lines and Balmer lines have the same profile, so although NGC1275 had an emission line spectrum similar to other class 2 Seyferts, the UBV colours are like those generally found for class 1 nuclei.

NGC1275 had, among Seyfert galaxies, a spectrum of unusually low excitation; He(II) and [Ne(V)] were weak whereas [O(I)] was strong⁵. Seyfert¹ noted that NGC1275 was of low excitation by comparing the intensities of the emission lines in that galaxy with those in NGC1068. The difference between the two was that, in NGC1275, the [O(II)] and [S(II)] lines were stronger and [O(III)] lines considerably weaker, while λ 4,686 He(II) was absent.

In view of these facts, Weedman⁶ in his list of known Seyfert galaxies did not classify NGC1275: "had it not been included in Seyfert's original study, NGC1275 would not have been grouped with the other galaxies called Seyferts today".

Optical variability of the nucleus of NGC1275 was suggested by Barnes⁷ and Peterson⁸, and confirmed by Selove⁹ and Canon *et al.*¹⁰. According to Penston *et al.*¹¹, the optical light curve of the nucleus showed irregular activity on a time scale of a month; Lyutyi¹² showed that the amplitude of the variations reached 1 or 1.5 mag. A literature search showed that all known optically variable galaxies are either Seyfert 1 galaxies or BL Lacertae objects. No Seyfert 2 galaxy is known to have a variable nucleus.

Stein *et al.*¹³ defined BL Lac objects as starlike objects or starlike galaxy nuclei having no emission lines. However, very faint emission lines ([O(III)] λ 3,727, [O(III)] λ 5,007 or both) have been observed in five BL Lac objects: BL Lac (ref. 14), AP Lib (refs 15, 16), 3C371 (ref. 17), PKS0521-36 (refs 18, 19) and 4C16.39 (ref. 20). It could be argued that there is a continuous transition between Seyfert 1 nuclei and BL Lac objects; however, the equivalent width relative to the non-thermal continuum of the broad Balmer emission lines in Seyfert 1 nuclei and QSOs is roughly constant^{21,22}, while such broad hydrogen lines have never been detected in any of the BL Lac objects, showing that if they are present at all, they are in any case much fainter than in the Seyfert 1 nuclei. Spectrophotometry of the nuclear region of NGC1275 through a 8 arcs aperture²³ showed that the equivalent width of [O(III)] λ 5,007 was about 5 Å and that the apparent magnitude inside the aperture was $B \sim 14$. From the spectrum of BL Lac by Miller and Hawley¹⁴, the equivalent width of the line can be estimated to be about 0.3 Å in a 3.3" $\times$ 4.0" aperture in September 1975, when the apparent magnitude of the nucleus was $B \sim 16$. Assuming that the total galactic visual absorption for this object is $A_v = 1.2$ mag²⁴, we found that the absolute luminosity of the nucleus of BL Lac (the redshift of which is $z = 0.070$ (ref. 25)) was about 2.0 mag brighter than that of NGC1275 (the red shift of NGC1275 was $z = 0.0176$; the galactic extinction, $A_v = 0.57$; reducing the luminosity of the nucleus of BL Lac by 2.0 mag and assuming a constant luminosity for

the emission lines, the equivalent width of [O(III)] λ 5,007 becomes 2.0 Å, which showed that the absolute luminosity of [O(III)] in NGC1275 is only 2.5 times larger than in BL Lac. But this number is very uncertain; also a sizeable fraction of the intensity of the emission lines measured can come from the extended system of filaments projected on the nucleus which should make smaller the equivalent width of the emission lines really associated with this nucleus.

Seyfert 1 galaxies are very faint radio sources, 3C120 being a notable exception. In the five first Markarian lists of galaxies with UV excess²⁶⁻²⁸, 28 Seyfert of class 1 were brighter than $V = 15.5$; only two of them had a detectable radio emission at 5,000 MHz (Mark 6 and Mark 231); but among the four galaxies with a featureless spectrum (Mark 80, 180, 421 and 501), three were strong radio sources, only Mark 80 being undetected²⁹ (Mark 80 is almost certainly a BL Lac object, as in addition to having a featureless spectrum³⁰, its nucleus is variable with an amplitude larger than $\Delta B = 2.1$ mag³¹). NGC1275 was identified with the strong radio source 3C84.0 (ref. 32). 3C84.0 had a compact component coincident with the nucleus of the galaxy³³ which was variable^{34,35}. The variations were not as fast as those observed in BL Lac or OJ287, but were comparable with those of PKS0735+17³⁶. Most BL Lac objects are radio variable, but radio variability is not a unique property of these objects, as 3C120 and many QSS are also radio variable.

The radio emission from BL Lac objects is polarised and usually variable, but the degree is modest ($\lesssim 3\%$). 3C84.0 has a very small amount of linear polarisation, certainly less than 0.5%³⁷⁻³⁹, but owing to the low degree of polarisation observed in the well known BL Lac objects, this is not a significant difference.

The nucleus is optically polarised, the degree of polarisation being as high as 5.1% in the UV band⁴⁰; both the degree of polarisation and the position angle are variable⁴¹. Substantial amounts of variation of polarisation have been detected on a time scale as short as one day. The polarimetric properties of NGC1275 are like those of the BL Lac objects rather than those of the Seyfert 1 nuclei⁴². NGC1275 shows very little polarisation at 2.2 μ m; this is in contrast with the large infrared polarisation in some BL Lac objects⁴³. However, so little is known yet about the infrared polarisation of these objects that strong polarisation may not be a characteristic property.

The Hubble classification of NGC1275 is uncertain; however, the luminosity profile shows that it is not a spiral but either an elliptical or an SO galaxy⁴⁴; moreover, the fact that it is the brightest galaxy in the rich cluster A426 (ref. 45) suggests that it is actually elliptical. Adams⁴⁶ has shown that most, if not all, of the Markarian or classical Seyfert 1 galaxies are spirals. On the other hand, Osterbrock *et al.*⁴⁷ have found that a number of elliptical radio galaxies have a Seyfert 1 nucleus (they call them 'broad-line radio galaxies'), but these elliptical Seyfert 1s have a much smaller spatial density than the spiral ones; moreover, all of them have the characteristics double radio structure, while the spiral Seyfert 1s are either radio quiet or associated with a compact radio source.

Each time it has been possible to determine the type of the underlying galaxy of a BL Lac object, it has been found to be elliptical: Mark 421 (1101+38)⁴⁸, AP Lib (1514-24)⁴⁹, Mark 501 (1652+39)^{48,50}, 3C371 (1807+69)¹⁷ and BL Lac (2200+42)^{51,52}. So if NGC1275 were a BL Lac object it will make more homogeneous both the Seyfert 1 class of galaxies and the BL Lac objects.

Assuming that the average V magnitude of the nucleus is 14.0 (ref. 53) and allowing for a galactic extinction $A_V = 0.57$ (ref. 54), the absolute magnitude of the nucleus is $M_V = -21.5$ ($H_0 = 50$ km s⁻¹/Mpc; the redshift of NGC1275 is $z = 0.0176$ ⁵⁵), that is the faintest known BL

Lac type nucleus but also the nearest.

It seems important to know whether it is possible to assign without ambiguity each galaxy with an active nuclei to a well defined class like Seyfert 1, Seyfert 2 or BL Lac objects, as such classifications may correspond to different physical phenomena and help us to understand them.

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Low energy cosmic ray erosion of ice grains in interplanetary and interstellar media

ICE grains can enter the Solar System from the local interstellar medium through which the Sun is moving¹ and from the release of icy grains by comets^{2,3}. It has been argued that charged grains can be removed from the Solar System due to the Lorentz force of the solar wind⁴. Without considering grain charge, Patashnick and Rupprecht^{5,6} have predicted, by theoretical and experimental work on ice sublimation rates, that the spectral absorption properties of the particles, together with the spectral distribution of solar radiation, leads to a quasi-stable size (~ 20 μ m) of interplanetary ice particles, independent of solar distances. We show here that the erosion of water ice from such grains by solar

energetic particles is likely to be the dominant process for determining a grain lifetime in the Solar System. Furthermore, we point out that energetic particle erosion of grain surfaces may be an important process in interstellar clouds.

We have shown that the 'sputtering' coefficients S_H of water ice by 0.5 and 1.5 MeV protons are 0.4 and 0.2, respectively⁷. This number is huge in comparison to the values of $\sim 10^{-3}$ – 10^{-2} calculated on the basis of classical sputtering theory⁸. These results are the first on the sputtering of frozen gases that have direct application to astronomical problems. The experiments also found for these energies that S_{He} is approximately 50 S_H and that the oxygen sputtering coefficient S_O is $\sim 50 S_{He}$. For electrons of approximately the same velocity as the ions, $S_e < 0.2 S_H$. On the basis of knowledge of the dependence on energy of the nuclear and atomic stopping powers, it can be expected that S_H (100 keV) $\geq S_H$ (500 keV) ≈ 0.4 .

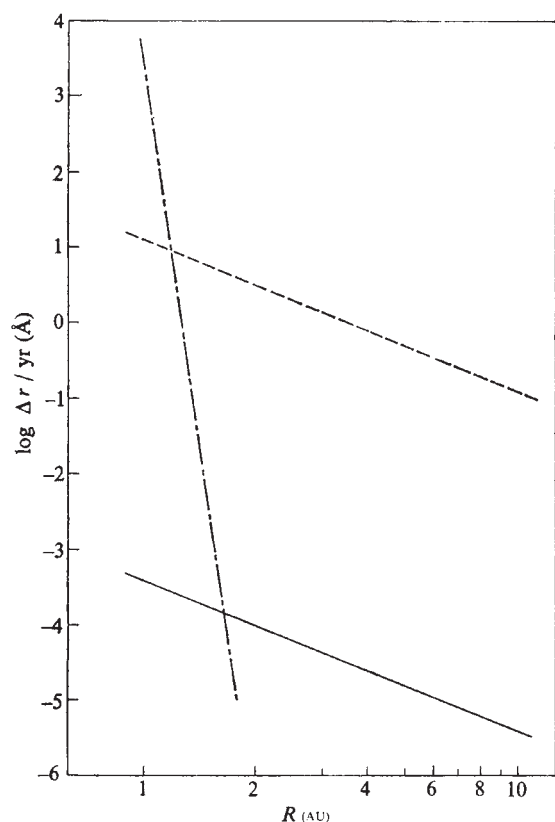


Fig. 1 Water ice erosion rate ($\Delta r/\text{yr}$, Å) as a function of heliosphere distance (AU). The solid line is for one typical solar flare particle event per year; the dashed line is for the solar wind. The dot-dashed line is for $\sim 20 \mu\text{m}$ particles extrapolated from the sublimation calculations and experiments of Patashnick and Rupprecht^{5,6}.

The sublimation studies^{5,6} show that the rate is a strong function of particle size. For example, at $R=1$ AU, particles of ~ 1 and $\sim 10^3 \mu\text{m}$ diameter experience sublimation rates $\sim 10^{-8} \text{ g cm}^{-2} \text{ s}^{-1}$ ($\sim 1 \text{ Å s}^{-1}$ radial shrinkage) whereas the sublimation rate for $20 \mu\text{m}$ particles is only $\sim 10^{-12} \text{ g cm}^{-2} \text{ s}^{-1}$ ($\sim 10^{-4} \text{ Å s}^{-1}$). The sublimation rate for $\sim 20 \mu\text{m}$ particles was found to drop very sharply with increasing distance from the Sun; between 0.5 AU and 1.25 AU, the rate decreased by $\sim 10^3$ per 0.25 AU.⁵ This radial dependence (Å yr^{-1}) is shown in Fig. 1.

We first consider a solar flare event, the energetic particles from which can be highly variable in flux level from one event to the next. A typical large flare event observed at 1 AU during the 20th solar cycle⁹ had a total proton flux greater than 1 MeV, $J > 1 \text{ MeV} \approx 10^{10} \text{ cm}^{-2}$. As the differential energy flux typically varies as $\sim E^{-2}$, the $J > 100 \text{ keV} \sim 10^{11} \text{ cm}^{-2}$. Averaged over a solar cycle, J_{He} (1 MeV/nuc)/ J_p (1 MeV) ~ 0.03 (ref. 9). As S_{He} (1 MeV)/ S_p (1 MeV) ~ 50 , the solar flare α particles can contribute as much as the protons to the interplanetary water ice

erosion. We assume that the solar particle density will decrease as R^{-2} (hence, we assume that transverse diffusion is negligible and that particles are not temporarily trapped between the Sun and an outer boundary). Considering both solar flare helium and protons and taking S_H (100 keV) = 0.5, the radial dependence of the water ice erosion rate (Å yr^{-1}) is shown in Fig. 1 for one typical event per year. We see that even for only one such event per year, the eroded layer thickness by $>100 \text{ keV}$ solar particles at 2 AU would be much greater than the shrinkage by sublimation of $\sim 20 \mu\text{m}$ grains at the same distance.

The solar wind particle flux is, of course, larger than the solar flare particle fluxes considered above, and is also a continuous source. No data exist on the erosion rates by solar wind energy protons. As the mechanism for erosion is still unclear⁷, an extrapolation to very low energies is uncertain. However, as an example, take S_H (1 keV) ~ 0.2 and $J(H)/J(He) \sim 10$. (It is reasonable to expect that S_H (1 keV) $< S_H$ (100 keV) because the particle electronic stopping power decreases below $\sim 100 \text{ keV}$). The erosion rate (Å yr^{-1}) is shown in Fig. 1 for a wind of density 10 protons cm^{-3} at 1 AU and a bulk velocity $\sim 400 \text{ km s}^{-1}$ (assuming the density is controlled by adiabatic expansion and thus decreases as R^{-2}). Hence, under the above assumptions, we see that the solar wind is the dominant mechanism for determining the lifetime of $20 \mu\text{m}$ particles outside $\sim 1.5 \text{ AU}$.

The sputtered water could, however, be ionised by solar photons and by charge-exchange with solar wind protons. The total ionisation rate is $\sim 10^{-6} \text{ s}^{-1}$ for H, H_2O , and O_2 and is $\sim 5 \cdot 10^{-7} \text{ s}^{-1}$ for O at 1 AU¹⁰. The mean distances reached by neutral atoms entering the solar system from interstellar space before ionisation is ~ 3 – 4 AU for hydrogen and oxygen¹¹. Ionised hydrogen and oxygen atoms could be accelerated by shock waves¹² or by transit-time damping¹³ in the interplanetary medium, producing energetic particles. Such energetic oxygen fluxes could form an 'anomalous' component to the low energy interplanetary cosmic ray population^{10,14}. The actual importance of this mechanism requires more detailed knowledge of the interplanetary grain population.

These considerations of interplanetary ice grains are applicable to considerations of interstellar grain lifetimes and surface characteristics^{15–17}. There are still many uncertainties about the nature of such grain surfaces and there is essentially no information at all on the cosmic ray fluxes of interest here. Most discussions of charged-particle impacts with grain surfaces are concerned with particle energies of a few hundred eV or less^{18,19}; yields of order unity are generally obtained from such estimates. A chemical exchange reaction ($\text{H}_2\text{O} + \text{H} \rightarrow \text{OH} + \text{H}_2$) has been applied to ice grains in H(II) regions¹⁹ and considerations of the dependence of the yield function on angle of incidence of the projectile have been applied to molecule formation in shock waves¹⁸. If these estimates are correct for these low energies, then the sputtering by higher energy cosmic rays (which have measured yields ~ 0.5) would be negligible because of the expected higher densities of the low energy particles in the interstellar medium.

Watson and Salpeter¹⁵ conclude from their several mechanisms for ejection of adsorbed molecules on grain surfaces, ultraviolet photons would be the most efficient. They note that grains of pure ice would be an exception. Hence, cosmic rays could be important for ejecting water molecules from grains into the interstellar medium. The cosmic ray erosion mechanism would tend to compete with the interstellar photon flux. Further, the cosmic rays could play an important role in ejecting other molecular species from grains in highly shielded (from ultraviolet fluxes) interstellar clouds, as experimental erosion results²⁰ for frozen volatiles such as NH_3 , CH_4 , CO_2 , O_2 , Ar, Xe, Kr, Ne by helium ions have shown even larger erosion coefficients for these species than for H_2O . Evaluation of the relative importance of the interstellar infrared flux and the cosmic ray flux at various energies in eroding water ice grain surfaces will require a detailed calculation such as that performed for the interplanetary medium but using the interstellar photon spectrum. In addition, reliable estimates of the low energy cosmic ray fluxes are necessary and are at present completely lacking.

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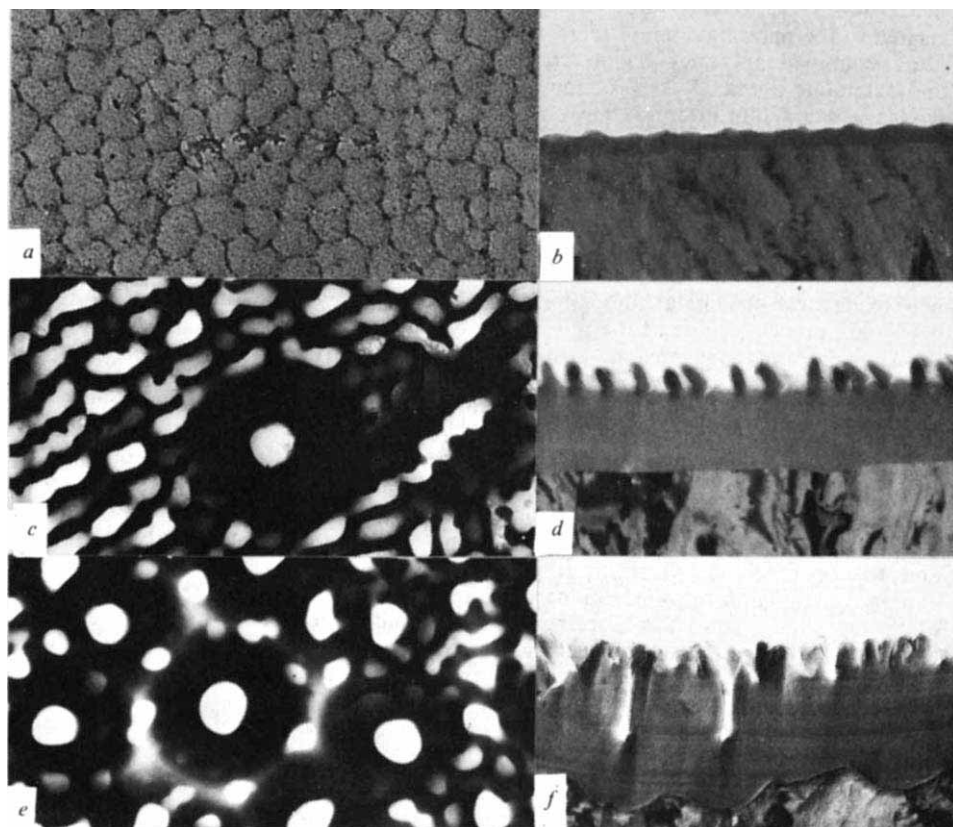
Nucleation and growth of porous anodic films on aluminium

Porous anodic films on aluminium are of considerable interest, not only because of their important practical uses in architecture and industry but also because of their novel regular morphology. The simple explanation involving field-assisted dissolution^{1,2} has now been extended and an additional mechanism involving direct loss of Al^{3+} ions to solution has been proposed³. Ultramicrotomy⁴ and ion beam thinning⁵ of films, together with transmission electron

microscopy and secondary ion mass spectrometry, have provided greater information on the film morphology and composition. Here we outline some of the more significant findings relating to the large-featured, regular films formed in phosphoric acid.

The initial, non-uniform growth of the anodic films formed in phosphoric acid at constant current density on aluminium (electropolished in a perchloric acid-ethanol mixture) is shown in Fig. 1. The micrographs show development of locally thicker film material to form approximately hexagonal cells (Fig. 1a, b). The cellular appearance is identical to that present on the metal surface after electropolishing and shows a similar metal grain orientation dependence. The thicker film material develops above ridges of metal initially present at the boundaries of the cells introduced by electropolishing. Formation of films, in nominally similar conditions, or substrates of a differently prepared but controlled surface cellular appearance (produced by forming a steady-state porous anodic film and dissolving the film in chromic/phosphoric acids to leave the scalloped substrate) confirms the preferential growth of material above the boundaries (Fig. 2). Likewise, where metal preparation is by etching, which produces a cellular network on the aluminium surface, or by mechanical polishing or scratching, which produce ridges, thicker film formation is again above the cell boundaries or ridges (in preparation). Further growth of the locally thicker material and the barrier layer, and a flattening of the initially ridged metal/film interface due to this more rapid growth at the ridges, are observed with continued anodising of an electropolished substrate (Fig. 1c, d). Pores are formed next within preferred cells, resulting in the now markedly scalloped appearance of the metal/film interface (Fig. 1e, f). With further anodising, the width of the scalloped metal beneath the barrier layer increases, as does the diameter of the pore base in order to maintain a uniform barrier layer thickness (dependent on voltage), until the scalloped regions intersect and create the steady-state porous film morphology with

Fig. 1 Transmission electron micrographs of the films formed on electropolished aluminium for various times at $50 A m^{-2}$ in 0.4 M phosphoric acid at 298 K. a, Stripped anodic film produced in 20 s, showing the non-uniform growth of the film with thicker material at the cell boundaries in an approximately hexagonal arrangement. b, Ultramicrotomed section of anodic film formed in 20 s, showing the scalloped nature of the metal/film interface and growth of thicker material above the ridges of metal. c, Stripped anodic film produced in 120 s, showing the elongated cellular appearance of the thicker film material (dependent on the metal grain orientation) and the onset of pore formation. d, Ultramicrotomed section of anodic film formed in 120 s, showing the thicker film material at the cell boundaries and the relatively flat metal/film interface. e, Stripped anodic film produced in 160 s, showing major pore development. f, Ultramicrotomed section of anodic film formed in 160 s, showing the scalloped metal/film interface beneath the developing pores. For a-f magnification $\times 80,000$.



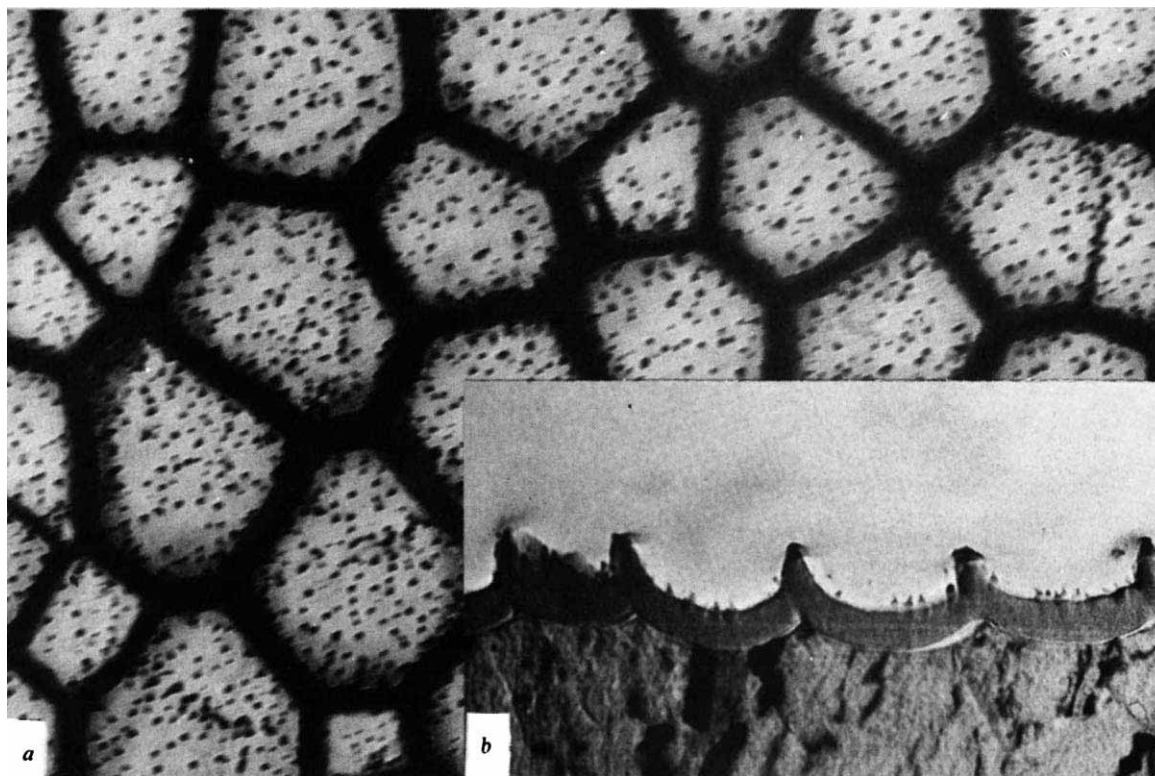


Fig. 2 Transmission electron micrographs of the film formed for 60 s on a preconditioned substrate at 50 A m^{-2} in 0.4 M phosphoric acid at 298 K. The preconditioned substrate was prepared by forming a steady-state porous film on the electropolished aluminium substrate in the above conditions for 10 min and removing the film in a mixture of boiling chromic acid-phosphoric acid. This produced an aluminium substrate with a cellular topography dependent on the steady-state anodising voltage (120 V). The nonhexagonal nature of the cells is related to the mechanism of porous anodic film formation. *a*, Stripped anodic film showing the development of thicker film material at the cell boundaries. The islands of locally thicker material within the cells have a population density of about $9 \times 10^{14} \text{ m}^{-2}$. *b*, Ultra-microtomed section of a showing the development of thicker film material above the ridges of metal. $\times 80,000$.

the major parameters dependent on the voltage.

In a parallel investigation secondary ion mass spectrometry was used to determine in-depth profiles of species related to the phosphate anion (data not shown). For all the films examined an inner region containing no phosphate and extending about 25–35% of the barrier layer thickness from the metal/film interface was observed. The results are in agreement with those determined previously for film growth in neutral phosphate solutions⁸.

Consideration of our studies suggests a model for initial film growth involving two simultaneous processes. (1) Growth by ionic migration through an existing film occurs, that is, both Al^{3+} and OH^- and O^{2-} are mobile and form new oxide near the metal/film interface. (2) Growth by a deposition process occurs at the film/solution interface, that is, Al^{3+} ions not consumed in growth by ionic migration are ejected at this interface to form hydrated ions in solution. (Direct solid state film formation through combination of ejected Al^{3+} ions with OH^- and O^{2-} plus phosphate is not thought to be a major process in the anodising conditions used, as the outer layer of film material is considered to be differently textured.) Solid alumina can form by deprotonation of the hydrated Al^{3+} ions, aggregation and thence precipitation from initially colloidal hydrous alumina; many film-forming anions can influence the rate of precipitation⁷. The phosphate species can stabilise a fine colloidal particle size distribution and delay the precipitation of impure, hydrous alumina. It is proposed that deprotonation of hydrated Al^{3+} ions and aggregation with some phosphate ions produces negatively charged colloidal particles, held in solution through the influence of the incorporated phosphate, which are deposited under the field above the thickening phosphate-free layer to produce an

outer layer of fine microcrystallites. Effective compaction of the deposited layer may occur by further deprotonation of the impure hydrated alumina by further reaction with some of the Al^{3+} ions ejected from the inner layer⁸.

During anodising the field strength is greater across the compact inner layer than across the impure film material, which probably varies from solid near the inner layer to gel-like material towards the outer surface of the film. The ridges of metal apparently act as more favourable sites for film growth and localised deposition. This may arise from a larger impurity-derived flaw density in the vicinity of the ridges compared with the more uniform regions between the ridges; within the limits of resolution, cracking of the film above the ridges has not been observed. If the effective current density is greater at the ridges than over the larger scalloped metal area, more rapid growth of the inner layer, a faster rate of Al^{3+} ejection and more localised deposition are all expected. Conversely, the efficiency of deposition is reduced in the lower current density regions because of the slower rate of Al^{3+} ejection leading to instability of the hydrous oxide sol and greater loss of colloidal material to the aggressive solution. Thickening of the film at the protuberances concentrates the current and film growth further into the thinner film regions in an attempt to obtain a uniform film thickness and maintain a constant field strength. The distribution of cell sizes enables the current density within the cells to vary, allowing the film material within the smaller cells to thicken more efficiently than that within the larger cells where deposition is hindered because of the lower rate of Al^{3+} ion ejection. The larger cells then probably become the sites at which pores develop because Al^{3+} ions are lost to solution through the low effective rate of deposition; local scalloping of the metal beneath the

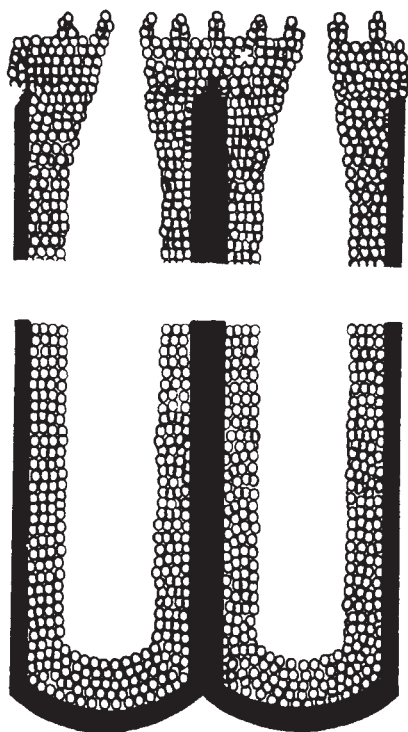


Fig. 3 Schematic diagram of the porous anodic film. The solid region represents the relatively pure alumina film material and the textured region depicts the microcrystalline film material with incorporated phosphate. The crystallites are not meant to be particularly regular and are probably of a size ≤ 2.5 nm. During anodising the field strength is greater across the compact inner layer than across the impure film material, which probably varies from solid near the inner layer to gel-like material in the vicinity of the pore base. The upper part of the diagram shows the appearance at the outer film/solution interface and the lower part of the diagram shows the steady-state morphology of the porous anodic film towards the metal/film interface.

barrier layer then proceeds in these preferred regions.

Steady-state film formation proceeds by steps (1) and (2) described above providing (3) transformation of the inner layer can occur. Transformation of the inner layer is also required to maintain the 2:1 ratio of phosphate-incorporated film material to relatively pure alumina in the barrier layer. The relative ease of transformation governs the steady-state anodising rate; this process may be identified as field-assisted dissolution or interface potential-assisted dissolution⁹, which is thermally enhanced. The H^+ and phosphate species move in the intercrystallite regions of the outer layer; there is no phosphate mobility under the field in the inner layer.

Figure 3 is a schematic diagram of the porous anodic film, showing the relatively pure alumina region and the phosphate-incorporated film material.

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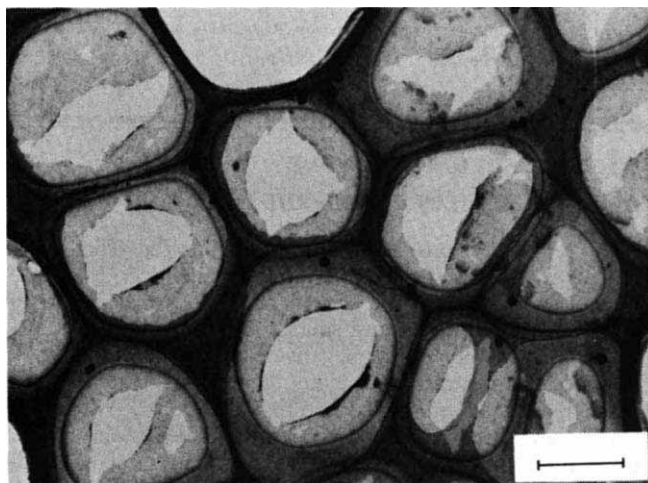
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Formation of films in hydrolysing ferric chloride solutions

FRYER *et al.*^{1,2} have postulated that two-dimensional polymeric structures may be formed in solution when metal-ion salts undergo self-hydrolysis before the precipitation of some hydrated crystalline oxides such as α FeO.OH, β FeO.OH, and $V_2O_5 \cdot H_2O$. They reported that these structures or films can only be seen by spraying a hydrolysing solution onto a mesh of asbestos fibres when the film appears suspended between the asbestos fibres supported on the copper grid. We describe here how we have repeated this work using 0.01 M FeCl₃ with both an asbestos mesh and a holey carbon support film (Fig. 1) and have obtained similar results. The films we have seen have large dimensions, of the order of several microns and of varying thickness depending on the drop size of the spray and the size of the mesh or hole. Assuming that the minimum thickness of the film is about the same as that of the partially hydrolysed spherical polymers seen by Murphy *et al.*³, that is, 10-50 Å then the equivalent spherical diameter (e.s.d.) of the films would be about 3,000 Å. Our observations of the films suggest that they can be much thicker than this.

If films of this order of size are actually present in these solutions, then it should be easy to centrifuge them out of the hydrolysing solution. A sample of the 0.01 M solution of FeCl₃ (pH 2.4) was centrifuged for 1 h at 60,000 r.p.m. in a L2.65B Spinco centrifuge using a TY65 head. The supernatant from the top half of the tube was sampled and examined. The same film as that seen in the original uncentrifuged sample was observed (Fig. 1). The solution collected from the bottom of the tube did not show a higher level of film, but there was a small amount of β FeO.OH depending on the age of the solution. The conditions of centrifugation should have sent to the bottom of the tube all particles with an e.s.d. greater than about 60 Å. These results suggested that the films were artefacts of the method of examination. This was confirmed by a further experiment in which FeCl₃ (0.01 M) was hydrolysed in H₂O (pH

Fig. 1 0.01 M FeCl₃ solution (pH 2.4) in the early stages (2 h) sprayed onto a holey carbon support film showing a fine grained film covering the holes in the carbon film. Scale bar, 1 μ m.



2.4) and D_2O ($pD \approx 2.4$) for 1 h. Examination of the D_2O and H_2O solutions separately showed that the same type of film material could be observed. A density gradient was then made from these solutions and centrifuged at 35,000 r.p.m. using an L2 Spinco centrifuge and a SW 50.1 head. The gradient was used to minimise convective and mechanical disturbance of any iron species gradient formed in the centrifugation. After centrifugation the solution in the centrifuge tube was carefully divided into four using a syringe. Each section was examined in the electron microscope and the same film material was found at all levels in the tube. Under the conditions of this experiment all material with an e.s.d. greater than 120 Å should have been removed from the top segment.

On close examination the film appears to be made up of small spherical polymeric molecules, of the type observed by Murphy *et al.*³, which are formed initially on partial hydrolysis of ferric salt solutions when base is added to them.

We believe the film forms when a solution of 0.01 M $FeCl_3$ solution is sprayed onto an asbestos mesh or micro-grid, due to the drying of droplets of solution caught in the holes of the micro-grid or between the asbestos fibres. Drying promotes the hydrolysis into the small polymeric molecules, as described by Murphy *et al.*, which on concentration form the film. Very thick films can be formed by placing a drop of solution onto an asbestos mesh or micro-grid and allowing it to dry. Similar artefacts of aggregates or clumps can also be observed when other colloid suspensions are sprayed or dropped onto carbon support films and allowed to dry.

Often the outline of a drop can be seen on the grid covering many holes in the support film. This would be expected if the film were produced as a result of the simple drying and concentration of the material in the droplets. Some micrographs show films which seem to contain crystallising species which could be formed due to the concentration changes on drying. We believe that crystal growth on ageing in solution results from polycations rather than from a film. The association between film and crystallising species reported by Fryer *et al.* could result from an entrapment in the film formed during drying.

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Mechanism of sputtering of solid surfaces by ion-impact

SECONDARY electrons and particles are ejected from a solid surface when it is struck by a beam of high energy (≥ 25 eV) ions. The majority of the ejected particles are neutral atomic and molecular species but a small proportion (typically 0.01% to 1%) carry a positive or negative charge. Two main theories have been advanced to explain the process; the 'hot spot' theory¹ and the 'momentum transfer' theory². We propose here a new theory in which bombarding species electronically excite the surface atoms of the target hence reducing the bonding energy of the species to the target.

Although qualitative agreement of calculated values and experimental results seems to have been obtained using a 'hot spot' theory at high ion-impact energies (≈ 10 keV) under controlled conditions¹, it is difficult to visualise a localised thermal equilibrium of sufficient temperature ($\approx 5,000$ K) being generated by an impacting ion with translational energy as low as 50 eV.

The large periodicity with atomic number observed in sputtering yields both for varying bombarding species³ at constant energy and varying target species⁴ cannot be accounted for using a momentum transfer theory, as only the first few collisions concern the primary ion and dependencies would be expected only on the primary ion's mass and momentum and the mass of the target species and not on their atomic numbers. These two theoretical mechanisms are, therefore, inadequate.

Our new theory suggests that the bombarding species electronically excite the surface atoms of the target. This electronic excitation may consist of promotion of electrons from bonding orbitals to weakly bonding, non-bonding or anti-bonding orbitals. The result is a reduction of the bonding energy of the species to the surface. These excited species may be either atoms or molecules of the target material itself or atoms or molecules of gases adsorbed on the surface of the target. If the bonding energy of a particular species to the surface becomes less than zero, then part or all of that species will be ejected from the surface, yielding a sputtered particle. Most of the species that leave the surface will be electrically neutral, but a small proportion will have become charged through the loss or gain of one or more electrons during the ejection process.

This mechanism for sputtering by ion-impact is similar to that for electron-impact desorption. Although the intensity of emitted ion current varies with electron energy more sharply in electron-impact desorption⁵ than does secondary ion current intensity with primary ion energy in sputtering¹, the shapes of the curves are basically similar at low energies (≈ 100 eV).

The theory explains qualitatively many of the features of ion and neutral emission during sputtering^{6,7}. It accounts for the observed angular distribution of sputtered particles⁸ (neutral and charged) from single crystals. The bonds from the sputtered atom or group of atoms were initially in a specific direction and the antibonding orbitals produced before ejection would also be in a specific direction (not necessarily the same), thus resulting in ejections in preferred directions.

The intensities of positive and negative ions emitted from a surface during sputtering are generally of the same order of magnitude⁹. This would be expected from the proposed theory where a large number of free electrons are not anticipated (unlike the hot spot theory of Andersen¹ which predicts more positive than negative ions). Anti-bonding electrons would have equal probability of leaving attached to the sputtered particle, or of remaining in the surface. For highly electropositive or electronegative atoms or groups of atoms, however, the bonds would be polarised before ejection, favouring the appropriate positive or negative emissions, respectively.

The emission of ions or neutral species which consist of a large number of atoms, such as are found in the sputtering of hydrocarbon-contaminated surfaces, is explicable because only a small proportion of the atoms comprising the adsorbed hydrocarbon species is directly bonded to the surfaces. If one of the electrons in one of the bonds is excited to sufficient energy to eject an atom, then the energy of the atom leaving the surface may be sufficient to break the remaining bonds holding the molecule to the surface, or break one or more bonds in the hydrocarbon and thus sputter a fragment. Similarly, the sputtering of dimers, trimers and so on from an atomically clean surface may be interpreted.

The 'pre-peak' observed by Bernheim *et al.*¹⁰ probably arises as a result of a fraction of the sputtered particles being electronically excited and metastable. After leaving the surface, some of these particles decompose, and some of the fragments would be repulsed back towards the surface, and their velocities after energy analysis seem to correspond to negative initial

values. Those repulsed forwards make a minor contribution to the high energy tail of the distribution.

The momentum transfer mechanism may be a secondary source of sputtered particles, particularly in the case of high energy primary ions and impact at oblique angles. The momentum transfer model accounts for the high energy tails observed in energy distributions of sputtered secondary particles¹¹.

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Structure of synthetic zeolite ZSM-5

ZEOLITE ZSM-5 (ref. 1) is a member of a new class of shape selective catalysts with unique channel structures which differ from the familiar large-pore faujasite and small-pore zeolites such as Linde Type A and erionite. They also possess unusual catalytic properties and have high thermal stability. We have determined their structure using model building, single crystal and powder X-ray data.

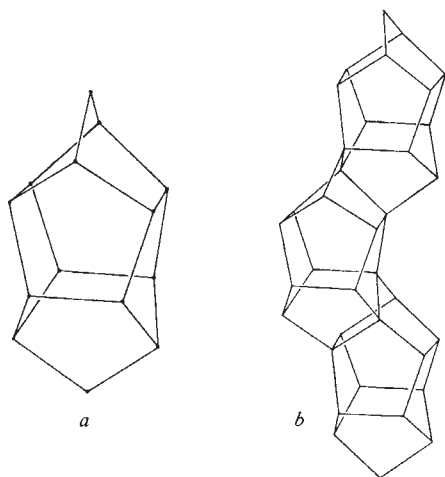


Fig. 1 Characteristic configuration (a) and its linkage within chains (b) in ZSM-5. These chains run parallel to [001]. Only T-atoms (Si, Al) are shown.

Methanol can be selectively converted to high quality gasoline over a ZSM-5 class of catalysts²⁻⁵. As methanol can be produced from coal, the ZSM-5 class of catalysts provides a key link for converting coal to gasoline. Moreover, this class of

zeolite catalysts has made possible the development of commercially significant petroleum and chemical processes, such as distillate dewaxing, ethylbenzene synthesis, xylene isomerisation and toluene disproportionation^{4,6}.

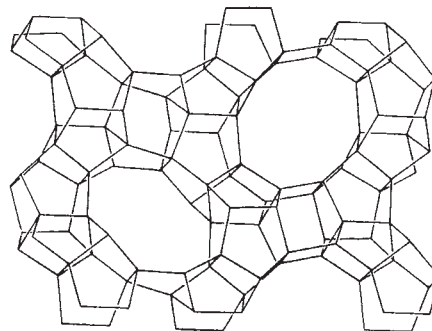


Fig. 2 Skeletal diagram of the (010)-face of the ZSM-5 unit cell. The x-axis is horizontal and the z-axis vertical. Oxygen atoms are not shown. The 10-membered ring apertures shown are the entrances to the straight channels which run parallel to [010].

The framework of ZSM-5 contains a novel configuration of linked tetrahedra shown in Fig. 1a and consisting of eight five-membered rings. This characteristic configuration with ideal 4,m2 (D_{2d}) symmetry has not so far been reported in any other zeolite. These ZSM-5 units join through edges to form

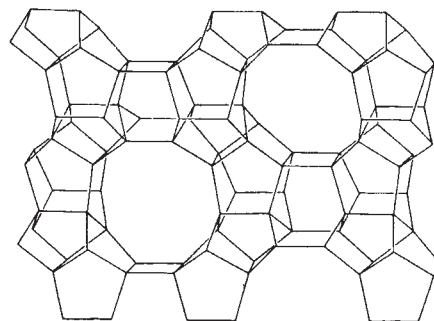


Fig. 3 Skeletal diagram of the (100)-face of the ZSM-5 unit cell. The y-axis is horizontal and the z-axis is vertical. Oxygen atoms are not shown. The nearly circular 10-membered ring apertures shown are the entrances to the sinusoidal channels which run parallel to [100].

chains as shown in Fig. 1b. The chains can be connected to form sheets and the linking of the sheets lead to a three dimensional framework structure. The chains extend along the z-axis. The sheets parallel to (010) and (100) are shown in Figs 2 and 3. The ZSM-5 framework can be generated by

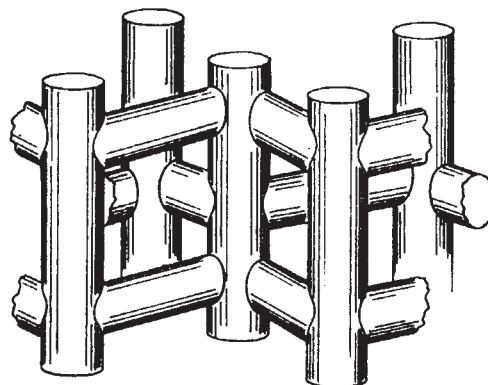


Fig. 4 The channel structure in ZSM-5.

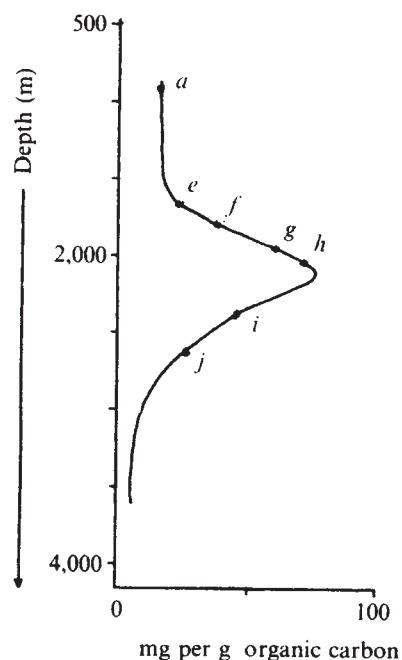


Fig. 3 Vertical variation of hydrocarbon concentration in the Cretaceous sediments from the Douala Basin, Cameroon, after Albrecht *et al.*⁶. a-j correspond with those indicated in Fig. 2.

I conclude that the compositional change of kerogens is shown by combination of vectors of the products on the C-H-O diagram and a large amount of hydrocarbons released from the kerogens reverse the direction of 'kerogen maturation' on the C-H-O diagram. This reverse pathway gives useful information on petroleum generation from kerogen.

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Oxygen in the Palaeoaquatic environment

A LARGE number of theories attempt to explain the 'apparently' very late and sudden diversification of life at the end of the Precambrian era either in terms of environmental changes (such as ocean chemistry¹, global temperature², and oxygen and ozone levels^{3,4}) or the time lag necessary for biological evolution⁵. It seems clear that no single mechanism can be solely responsible for this sudden leap forward in evolution; the origin of multicellular life was almost certainly in response to several forcing mechanisms. The model we present here does not attempt to resolve which parameter was the most important, but seeks to emphasise the vital role of adequate oxygen levels in the early marine environment which must be considered as a further vital condition for life diversification.

Abundance of oxygen has often been cited as a criterion for the diversification of life around the beginning of the Phanerozoic era. Some early theories suggested either that oxygen levels were not high enough to support complex respiring metazoa until the end of the Precambrian³, or that Precambrian life was restricted to localised environments by the need to 'hide' from the destructive ultraviolet (UV) radiation not then absorbed by an ozone shield in the stratosphere⁴. Both these assumptions have been shown to be incorrect by recent geological and biological evidence⁵. It is now established that free oxygen existed in the Precambrian atmosphere and thus it seems reasonable to suppose at least some protection from UV radiation by ozone was afforded to life before the Phanerozoic. However, the vital importance of abundant supplies of oxygen to the origin of complex lifeforms should not therefore be dismissed. We believe that it is possible to identify the most likely region on the Earth where this diversification would occur, by examining oceanic oxygen levels determined from the average position of the pycnocline.

In the palaeocean, stratification was a fairly permanent feature⁶. In the low latitudes this was due to temperature (thermocline) and in higher latitudes to salinity (halocline). The depth of the pycnocline can be modelled by considering the thermal processes in any water body. For an individual lake this can be accomplished in great detail^{8,9} and similar complex models^{10,11} are available to describe oceanic stratification. However, it is convenient to use a simplified latitudinal model described recently¹² to study the effects on life development of the pycnocline depth. This model was designed primarily to predict latitudinal change of pycnocline depth in regions where the variation in solar energy is the most important forcing parameter. The model is thus only strictly applicable at middle or high latitudes

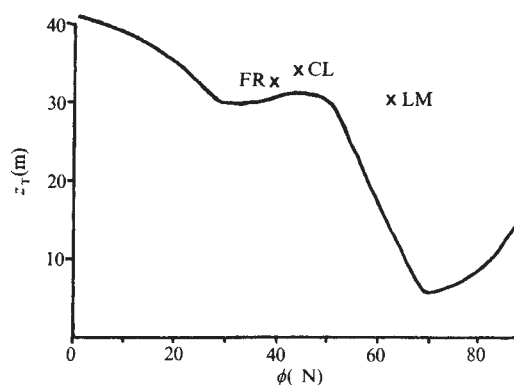


Fig. 1 Pycnocline depth as a function of latitude, 600 Myr BP FR, Fontana Reservoir; CL, Cayuga Lake; LM, Lake Mjosa.

but has been used here (for continuity) at low latitudes as a first order approximation. At low latitudes the mixing process is primarily wind controlled¹³ and the pycnocline depth correspondingly larger¹⁴. Using this model, the mean pycnocline depth, z_T , is expressed as a function of latitude by

$$z_T = 0.30 \times 10^{-8} F_c U \text{ (m)} \quad (1)$$

where U is the wind velocity and F_c the total incoming solar flux absorbed by the water during the spring-summer warming period. Both U and F_c are functions of latitude, ϕ . We have used this simple model of stratification to attempt to assess the likely zones for life diversification at the beginning of the Phanerozoic, 600 Myr ago.

Although global wind patterns may have changed during the evolution of the Earth, a reasonable first approximation

is to assume that $U(\varphi)$ was the same as present day values. Determination of F_c depends on knowledge both of the incident solar flux at the top of the Earth's atmosphere and of atmospheric evolution. This model can be applied unreservedly to more recent geological times, when the atmospheric state was almost identical to that of the present day (certainly the past 1,000 Myr). Although recent developments in stellar modelling reveal small scale fluctuations in the solar luminosity¹⁵ a reasonable approximation for this model is given by a standard stellar model¹⁶ in which the Sun's luminosity is assumed to have increased by about 43% over its lifetime. Figure 1 is derived from equation (1) to illustrate the pycnocline depth at 600 Myr ago, when the solar luminosity was about 96% that of the present day value. (Modern observed pycnocline depths, indicated in Fig. 1, are several per cent greater than the values predicted for 600 Myr ago). The implication of this graph depends upon the fact that the pycnocline acts as a barrier to transfer processes. Oxygen released by photosynthesis is retained in the water above the pycnocline and its concentration is thus determined by the depth of the upper layer. Diffusion of oxygen across the water-air interface is governed by the oxygen gradient and in the palaeoatmosphere-ocean system would lead to a transfer of oxygen from the water and into the atmosphere. If the layer of water above the pycnocline is too deep, then the oxygen (released by photosynthesis in the upper levels and diffused evenly throughout this depth) has a low concentration.

Figure 1 seems to indicate that only certain latitudinal zones would be hospitable for the early evolution of multicellular life. It is immediately evident that (presuming a fairly homogeneous distribution of single-cell photosynthetic organisms) at latitudes lower than about 50° the pycnocline is so deep that only low oxygen concentrations would ensue. At higher latitudes, however, the pycnocline is fairly shallow and thus we expect high oxygen concentrations suitable for the evolution of complex respiring organisms. It should also be noted, however, that towards polar regions the average temperatures would be expected to be too low for the establishment of multicellular life. Thus we predict that multicellular life may be expected to have originated in latitudinal zones between approximately 50–70° N and S, forced by the abundance of oxygen in the upper ocean.

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Zonation of supracrustal relics in the Archaean of Sierra Leone, Liberia, Guinea and Ivory Coast

THE West African Craton¹ contains a distinct province of Archaean rocks which outcrop in Sierra Leone, Liberia, Guinea and Ivory Coast. Rb-Sr whole rock isochron ages fall between 2,600 and 2,800 Myr BP (refs 2, 3). This paper presents a comparative stratigraphic interpretation of supracrustal relics in this region, based on new maps of three of the supracrustal belts in Sierra Leone, which I made whilst employed by the Geological Survey of Sierra Leone, and a re-examination of published maps and memoirs in the light of field experience. No previous stratigraphic interpretation has been made of the Sierra Leone belts and no previous comparison has been made of supracrustal belts within the West African Archaean. Supracrustal relics have been mapped over a large area. When comparisons are made they seem to fall into two groups on the basis of their size, stratigraphic thickness, lithologies, metamorphic grade and geographical distribution (Fig. 1). First, in Sierra Leone to the west of this region there are large schist belts (up to 130 km long) with thick successions (up to 6.5 km) metamorphosed to amphibolite grade. Banded iron formation is a relatively minor lithology. Second, in southeastern Sierra Leone, Liberia and western Ivory Coast there are small schist relics (maximum 40 km long) with thinner stratigraphic successions (rarely greater than 1 km) in which banded iron formation is dominant and gabbro anorthosite complexes are minor; these rocks were metamorphosed to granulite grade. Geochronological evidence suggests that the two groups of supracrustal rocks are contemporaneous and it is proposed here that they reflect a primary zonation of the Archaean in southern West Africa.

The stratigraphy of the larger, Sierra Leonian schist belts has been subdivided into a lower ultramafic, a middle mafic and an upper sedimentary group (Fig. 2). The principal rock types in the ultramafic group are tremolite-chlorite, talc-chlorite and anthophyllite schists and serpentinite interbedded with amphibolite and more rarely metasediments. Some ultramafic schist horizons represent layered sills⁷, and thinner bands of ultramafic schist in the lower part of the Nimini belt succession may represent ultramafic lavas; they are interbedded with amphibolite, fuchsite-bearing metasediments and ultramafic tuff. No pillows or relict spinifex textures have been observed. The mafic group is made up entirely of amphibolites which vary in grain size, show pillows, vesicular and amygdaloidal structures indicating that the original basaltic pile was composed of pillowed and massive lavas and intrusive sills with chilled margins. Amphibolites from the Kambui belt are olivine tholeiitic in composition⁸. The most common lithological types in the sedimentary group are greywacke turbidite and quartzite with minor amounts of fuchsite quartzite, conglomerate, meta-chert and banded iron formation. Conglomerates generally contain locally derived pebbles, although clasts of low potassium granite occur in the Sula mountains⁹ and the Gori Hills indicating the proximity of continental crust during the evolution of these belts. Facies variation is common within individual belts, making detailed intrabelt correlation difficult. The metamorphic grade of these belts is constant across the craton; the assemblage andesine-hornblende in the amphibolites suggests mid-amphibolite facies of metamorphism.

The smaller schist relics of southeastern Sierra Leone, Liberia and western Ivory Coast have much less regular stratigraphies^{4,5,10,11} (Fig. 2). Metasediments dominate the successions, although small amounts of mafic and ultramafic rocks are present. Banded iron formation is very common and characterises these smaller belts; quartzite, greywacke and pelitic schist are also common. Metamorphic grade is variable both within belts and between belts from greenschist to granulite facies; sillimanite is common and kyanite is recorded¹². Gabbro-

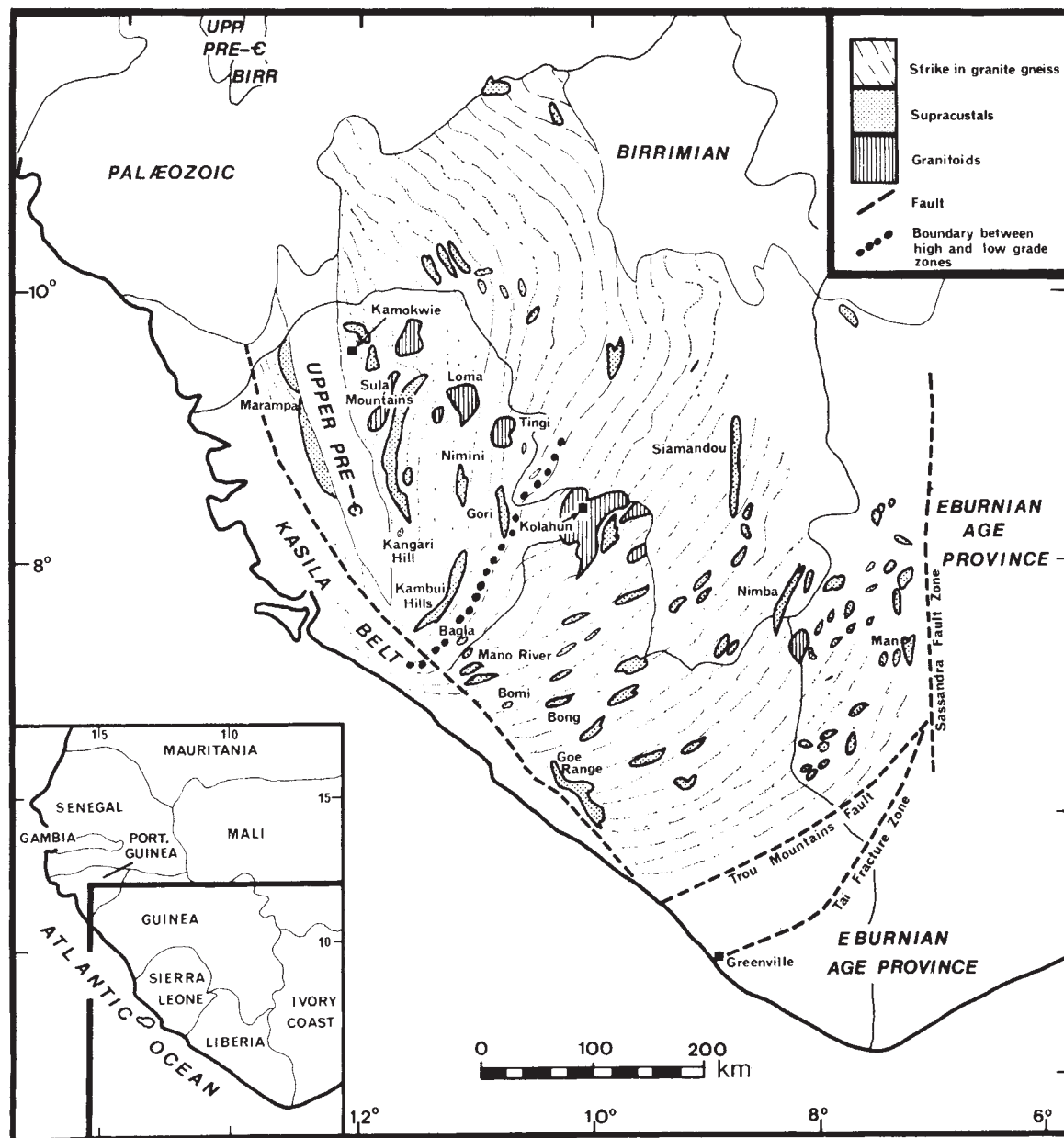


Fig. 1 Distribution of supracrustal relics in the Archaean of Sierra Leone, Guinea, Liberia and Ivory Coast, and the proposed boundary between high and low grade zones.

anorthosite complexes are described from southeastern Sierra Leone¹³ cutting banded iron formation of the smaller schist relics (Fig. 3). A similar association is reported by Tagini¹⁴ from the Man charnockite complex in western Ivory Coast, where norite and anorthosite are associated with small banded iron formation and basic granulite bodies. Ultramafic rocks are also reported associated with banded iron formation in Liberia¹². The Nimba and South Kambui belts seem to represent an intermediate type of succession both in their thickness (2 km), location and lithology (Figs 1 and 2). The South Kambui belt has a lower sedimentary unit, primarily quartzites; the Nimba belt is similar to the larger belts of Sierra Leone and yet has a very thick (500 m) banded iron formation unit.

The Marampa and Kamokwie belts in western Sierra Leone are anomalous in that they seem to have thin successions dominated by banded iron formation¹⁵, but they may not be the same age as the other belts.

Supracrustal rocks from both high and low grade zones have been folded into a series of tight anticlines and synclines, showing a similar structural style across the craton. Sedimentary

structures and pillows are rare in the low grade zone and have not been reported in the high grade zone. This may reflect a higher degree of deformation in the high grade zone, which may account for some of the difference in stratigraphic thickness between the two zones. There is also the possibility that the thick successions in the low grade zone represent piles of thrust sheets, but no thrusts nor any obvious stratigraphic repetitions have been found.

A third zone is the north-west-south-east trending Kasila belt (Fig. 1), which flanks the main craton and contains highly deformed banded iron formation and gabbro-anorthosite complexes metamorphosed to granulite facies¹⁶, an identical association to that found in Liberia and Ivory Coast. Structures can be traced from the craton into the Kasila belt and an analogy has been made with the Limpopo mobile belt which flanks the Rhodesian and Kaapvaal cratons¹⁶. It is important to note, however, that unlike the Limpopo belt the Kasila belt is not the sole site of high grade, small schist relics (see ref. 17) and probably represents a late Archaean shear zone into which high grade schist relics have been rotated and dextrally displaced.

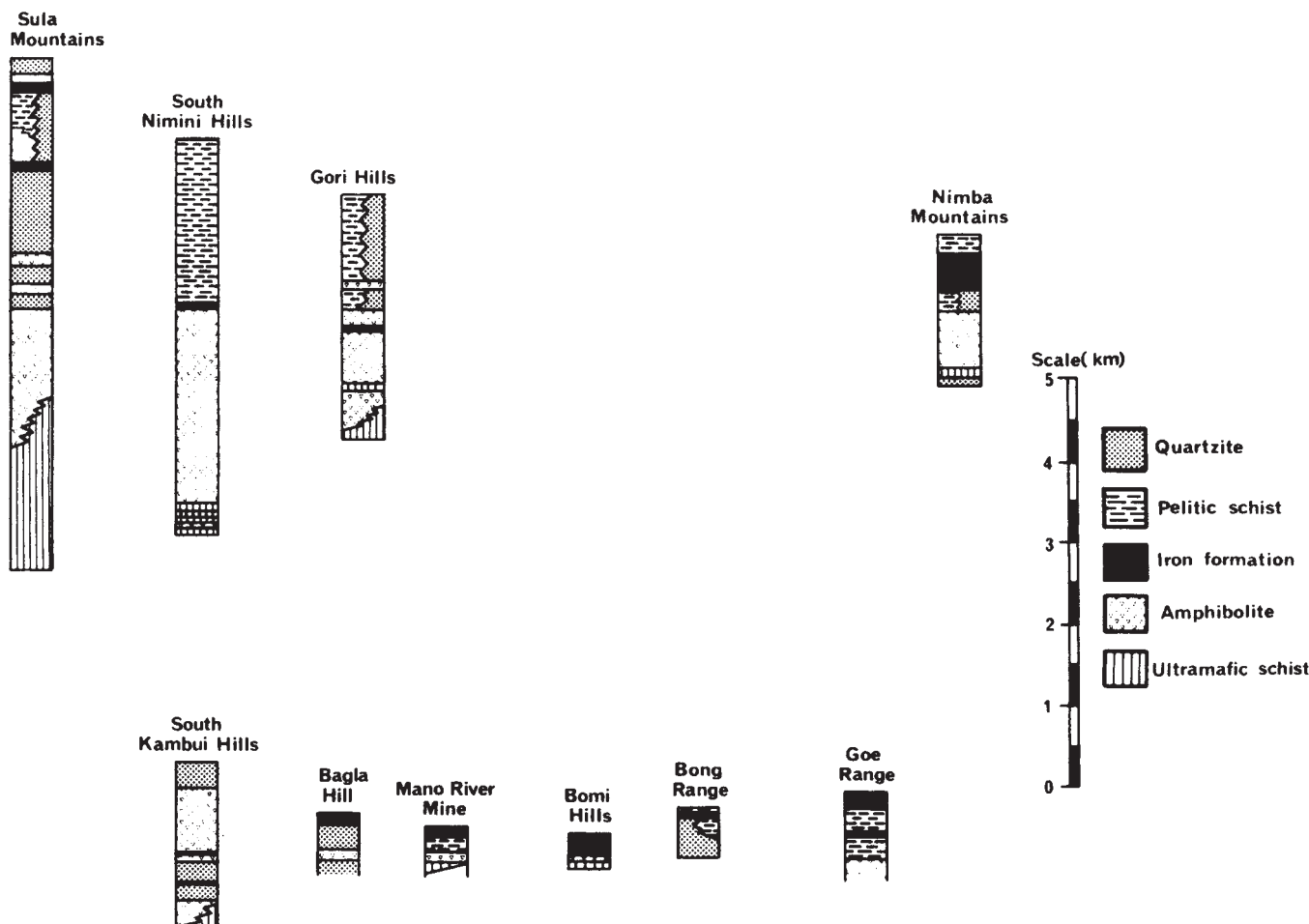


Fig. 2 Comparative stratigraphic sections for the supracrustal belts across the craton, showing the approximate spatial relationships between the belts. (Based on my unpublished field work, refs 4–6 and compilations from refs 9–11).

It has been suggested^{13,18}, because of their difference in metamorphic grade, that the two types of supracrustal belt are of different ages. Whilst no detailed radiometric determinations have been made on the supracrustal belts Rb–Sr whole rock ages on the associated quartzofeldspathic gneisses all fall within the age range 2,600–2,800 Myr BP (refs 2, 3) suggesting that the two groups of supracrustal belts are coeval.

A comparison can be made with supracrustal belts in other Precambrian terrains. The larger belts with a thick succession dominated by greywacke turbidites, a lower metamorphic grade (amphibolite facies) relative to the smaller belts and a regular stratigraphy with a high proportion of basic volcanic rocks and ultrabasic rocks of possible extrusive origin are comparable with greenstone belts sited near the edge of the Kaapvaal and Rhodesian cratons close to the Limpopo belt, where they are metamorphosed to amphibolite grade¹⁹. In the smaller granulite facies belts the successions are thin and the stratigraphies varied, the dominant rock types are banded iron formation, pelites and quartzites; basic rocks are represented by intrusive gabbro complexes. This association is typical of high grade gneiss terrains in many parts of the world²⁰.

In the West African Archaean, therefore, field evidence suggests that there is structural and metamorphic continuity between high grade and low grade zones of the same age, which predates the Kasila mobile belt. The type of sediments and proportion of basic volcanics in the two zones imply a marked difference in sedimentary environment and tectonic setting; in the lower grade areas deep water conditions and less stable tectonic conditions prevailed, whereas in the higher grade zones the dominant sedimentary environment was shallow water and tectonic conditions were more stable. The difference in

metamorphic grade reflects the continued contrast in crustal environment and implies a fundamental difference in tectonic setting between the two zones.

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α -Hydroxycarboxylic acids in the Murchison meteorite

A VARIETY of indigenous organic compounds have been detected in carbonaceous meteorites¹⁻⁶, and of these, the amino acids have been the most extensively studied^{1,7-11}. In addition to the amino acids commonly found in the proteins of terrestrial organisms, a large number of nonprotein amino acids have also been detected⁸. Moreover, the amino acids in meteorites exist as a racemic mixture, demonstrating their abiotic origin^{1,8-10}. The general abundance pattern of amino acids in the Murchison meteorite is similar to that obtained in a typical prebiotic electric discharge experiment¹². The mechanism of formation of amino acids in the electric discharge is by way of the Strecker synthesis, that is, the reaction of hydrogen cyanide, aldehydes and ammonia in aqueous solution, producing amino nitriles, which yield amino acids following acid hydrolysis^{13,14}. In the electric discharge experiment, in addition to amino acid formation, the corresponding α -hydroxycarboxylic acids (hydroxy acids) are also produced by the reaction of hydrogen cyanide and aldehydes (the cyanohydrin synthesis). The ratio of hydroxy to amino acids depends on the amount of ammonia present in the system^{13,14}. At high ammonia concentrations, amino acids are preferentially synthesised, whereas if ammonia is absent, only hydroxy acids are produced. Thus, the detection of hydroxy acids in carbonaceous meteorites would provide evidence in support of the hypothesis that the Strecker mechanism is responsible for the natural extraterrestrial synthesis of amino acids found in meteorites. In addition, the ratio of hydroxy to amino acids would provide some indication as to the ammonia content of the environment in which these compounds were synthesised. We report here the presence of seven α -hydroxycarboxylic acids in the Murchison meteorite, a type II carbonaceous chondrite.

We give here only a general description of the analytical procedure used in the detection of the hydroxy acids (a more detailed discussion of the individual steps will be reported elsewhere). Throughout the analysis, special precautions were taken to minimise terrestrial contamination of the sample. All water was deionised, then doubly distilled. Hydrochloric acid was doubly distilled as the 6M azeotrope. All glassware was cleaned by first soaking overnight in 10% sodium hydroxide, followed by washing with concentrated nitric acid, then thorough rinsing in doubly distilled water.

Two pieces of the Murchison meteorite were used (obtained through Dr D. MacDougall, UCSD, from Dr E. Olsen, Field Museum, Chicago). Both samples were carefully cleaned of the fusion crust and then the outer 1–2 mm of material was removed, using a hand-held grinding tool. The remaining material was then crushed with an acid-cleaned agate mortar and pestle to <0.5 mm in diameter. One portion (15.6 g) was transferred into clean Pyrex glass tubes, and ~10 ml of doubly distilled water was added. The test tubes were then sealed under vacuum and heated at 100 °C for 20 h. The second portion (3.9 g) was extracted with water in a similar manner, then the water extract was evaporated and the residue taken up in ~10 ml of 6 M HCl and hydrolysed at 100 °C for 20 h. In addition to the two extracts from the Murchison meteorite, a sample from an electric discharge experiment (specifically, a portion of the cation-exchange column water wash following acid hydrolysis^{12,15}, was also obtained.

The sample extracts were evaporated individually to ~1 ml total volume and then desalted on cation exchange resin (BIORAD AG50W-X8, H⁺). The water wash was collected and concentrated to ~0.5–1 ml and then diluted to ~10 ml. This solution was then carefully transferred to

an anion exchange column (BIORAD AG1-X8, OH⁻). The anion column was washed with water, then eluted with 0.25 M HCl. The eluate was collected, concentrated to a few tenths of a ml, mixed with 1 ml of 1 M HCl, and then coated on 3 g silica gel. The silica gel was slurried with 20 ml chloroform and transferred to the top of a column containing 10 g silica gel, pretreated with 4 ml of 1 M HCl. The column was first washed with chloroform and then eluted with 125 ml of 10% *n*-butanol–chloroform, followed by 200 ml of 40% *n*-butanol–chloroform. (The silica gel chromatography procedure was adapted from Bulen *et al.*¹⁶ and was necessary to separate the hydroxy acids from other compounds which interfered with the gas-chromatographic analyses. Two fractions were collected in order to separate glycolic and α -hydroxy-*n*-butyric acids, which have identical gas-chromatographic retention times.) The two fractions from the silica gel column were evaporated to dryness and the methyl esters prepared by derivatisation with diazo-methane in ether. Analysis of the methyl esters was accomplished on a Hewlett-Packard 5711A gas chromatograph

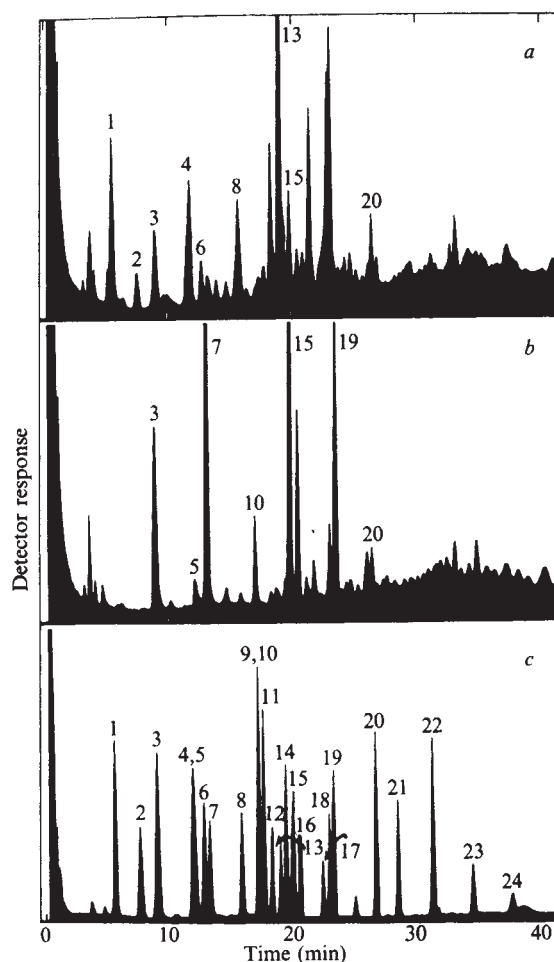


Fig. 1 Gas chromatogram of methyl esters of hydroxy and dicarboxylic acids in *a*, 10% *n*-butanol–chloroform fraction, and *b*, 40% *n*-butanol–chloroform fraction of the unhydrolysed water extract of a 15.6-g sample of the Murchison meteorite; *c*, standard mixture of hydroxy acid methyl esters. Gas chromatography: 2 mm $\times$ 1.8 m glass column packed with 7.5% Carbowax 20M TPA on 120/140 Chromosorb W AW/DMCS; temperature programmed from 80 °C to 180 °C at 4 °C min⁻¹ after 8 min. Compound identification: 1, α -hydroxyisobutyric; 2, α -hydroxy- α -methylbutyric; 3, lactic; 4, α -hydroxy-*n*-butyric; 5, glycolic; 6, α -hydroxyisovaleric; 7, oxalic; 8, α -hydroxy-*n*-valeric; 9, α -hydroxy- β -methylvaleric; 10, malonic; 11, α -hydroxyisocaproic; 12, fumaric; 13, methylsuccinic; 14, α -hydroxy-*n*-caproic; 15, succinic; 16, mesaconic; 17, maleic; 18, citraconic; 19, glutaric; 20, adipic; 21, tartaric; 22, malic; 23, α -hydroxyglutaric; 24, α -hydroxyglutaryl- γ -lactone.

equipped with a flame ionisation detector. The samples were also analysed on an HP5710/5930/5933A gas chromatograph-mass spectrometer-computer data system.

The results of the gas-chromatographic analyses of the unhydrolysed water extract fractions, together with a standard hydroxy acid mixture, are shown in Fig. 1. Similar results were obtained for the acid-hydrolysed extract. On the basis of their retention times, various hydroxy acids seem to be present. To confirm the presence of these compounds, the methyl ester derivatives were analysed by combined gas chromatography-mass spectrometry. The mass spectra and the retention times for the various hydroxy acids detected in the Murchison meteorite were found to be identical to those obtained for authentic compounds. These analyses demonstrate conclusively the presence of the following acids: glycolic, lactic, α -hydroxy-*n*-butyric, α -hydroxy-isobutyric, α -hydroxy-*n*-valeric, α -hydroxyisovaleric and α -hydroxy- α -methylbutyric. In addition to the hydroxy acids, several dicarboxylic acids were detected, which is in agreement with the results obtained by Lawless *et al.*⁵

The concentrations and relative abundances of the hydroxy acids are summarised in Table 1. Individual concentrations of the hydroxy acids for the acid-hydrolysed water extract were less than those shown for the unhydrolysed fraction, although the relative proportions remained the same. We attribute this decrease in concentration following acid hydrolysis to either acid-catalysed decomposition or the fact that the hydroxy acids may be more volatile in strong acid than in water. It is interesting to note that the hydroxy acids in the meteorite exist primarily in a free or water-hydrolysable state, whereas the amino acids exist in some kind of polymeric form and are released only on hydrolysis in 6 M HCl (ref. 7).

The water-extractable hydroxy acids are present in the Murchison meteorite at about the same concentrations as have been found for hydrolysable amino acids^{7,17}. Also, the hydroxy acids exhibit the same general abundance pattern displayed by the amino acids, with the exception that the relative abundance of glycolic to lactic acid is much less than the relative abundance of glycine to alanine. This may be attributed to the fact that glycolic acid is more volatile than the other hydroxy acids¹⁷. The general spectrum of hydroxy acids in the meteorite also was found to resemble closely that observed in the sample from the electric discharge experiment. (A detailed discussion of these latter results will be presented elsewhere.) These comparisons strongly suggest that the hydroxy and amino acids in the Murchison meteorite and those in the electric discharge experiment were formed by similar mechanisms, namely, by the Strecker-cyanohydrin synthesis.

To demonstrate that the hydroxy acids we have detected in the Murchison meteorite were indeed indigenous and not terrestrial contamination, we determined the enantiomeric composition of some of the hydroxy acids. Diastereomeric derivatives were prepared by reacting the hydroxy acid methyl esters with *N*-trifluoroacetyl-L-prolyl chloride (TPC). Analyses of the diastereomeric derivatives were carried out by gas chromatography on a 2 mm $\times$ 3.4 m glass column packed with 7.8% SP-2250 coated on Chromosorb W AW/DMCS. If the hydroxy acids in the meteorite were of abiotic origin, then they should exist as racemic mixtures, as is the case for the amino acids¹. The results of our enantiomeric analyses (Table 1), show that the hydroxy acids are essentially racemic. These data indicate that the hydroxy acids we have detected in the Murchison meteorite are indigenous.

Our detection of several hydroxy acids in the Murchison meteorite provides new evidence that the Miller-Urey electric discharge synthesis of amino and hydroxy acids is a reasonable model for the prebiotic formation of these

Table 1 Hydroxy acids in the Murchison meteorite

Hydroxy acid	Amount ($\mu\text{g} \times \text{g}^{-1}$)	Mole ratio (relative to glycolic)	Enantiomer ratio (D/L)
Glycolic	1.6	1.0	—
Lactic	5.9	3.0	0.92 $\pm$ 0.1
α -Hydroxy- <i>n</i> -butyric	2.3	1.0	0.82 $\pm$ 0.1
α -Hydroxyisobutyric	2.2	1.0	*
α -Hydroxy- <i>n</i> -valeric	1.6	0.6	(0.91 $\pm$ 0.3) [†]
α -Hydroxyisovaleric	0.5	0.2	(0.93 $\pm$ 0.3) [†]
α -Hydroxy- α -methylbutyric	0.5	0.2	*
Total	14.6		

*No TPC derivatives were formed because TPC does not react with tertiary alcohols.

[†]D/L ratio corrected for unknown compound partially overlapping 'L' peak.

compounds. Moreover, the abundance of amino and hydroxy acids in the Murchison meteorite can be used to estimate some of the chemical conditions in the extra-terrestrial environment where these compounds are synthesised. These calculations will be presented elsewhere.

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Resonant wing systems in the Australian whistling moth *Hecatesia* (Agaridae, Lepidoptera)

DARWIN¹ described the sound made by the forewings of the nymphalid *Ageronia feronia* as a click of a spring catch on a toothed wheel. Descriptions of the sounds produced by the Agaridae, which have similarly modified forewings, have repeatedly invoked the rubbing of either the antennae or foretibia on the ribbed forewing^{2,3}. Nicholson⁴ suggested that the production of sound in the whistling moth *Hecatesia* was percussive, with the knobs of each forewing (castanets) striking each other at the top of the flight stroke. I have confirmed the existence of such a percussive system in two species of *Hecatesia*. Recordings of *H. exultans* Walker and *H. thyridion* Feisthamel from the coastal sand plain of Western Australia revealed two

patterns of sound corresponding to the respective flight behaviour of the two species.

The sounds of both species were recorded in the field using a Nagra IV-SJ recorder with a Bruel and Kjaer 0.5-inch microphone. This provided good free-field conditions; however, because of the random flight pattern of *H. thyridion* a 50-cm parabolic reflector was used. Possible artefacts due to Doppler effects in flight were undetectable as the frequency emitted in consecutive calls remained stable.

The song of *H. exultans*, which is emitted during periods of rest consists of a series of rapid clicks with an interval of approximately 5 ms. The clicks are usually grouped into bursts of 10–15 pulses but this grouping periodically breaks down into a continuous pulsed train (Fig. 1a). The wings, when held in the sound-producing position, form an arch over the abdomen, bringing the two castanets close together over the mid-line. The castanets in this position have a small make-and-break distance, and slight muscular contraction causes them to strike. The castanets appear to be supported by elastic membranes (Fig. 2). This system produces a song consisting of impacts or 'bounces' of decreasing amplitude (Fig. 1c). A similar elastic recoil can be evoked more easily in the laboratory with freshly killed specimens so that, if the wings are allowed to strike in a near natural

Fig. 1 Analyses of the calls of two species of *Hecatesia*. a, *H. exultans* showing grouped and continuous pulse train; b, *H. thyridion*; c, single wing percussion with two bounces, *H. exultans*; d, wave form of one pulse, *H. exultans*; e, *H. thyridion*; f, frequency spectrum of an isolated wing of *H. exultans* struck mechanically; g, frequency spectrum of the natural call of *H. exultans*; h, *H. thyridion* (Tektronix 5L4N spectrum analyser—linear).

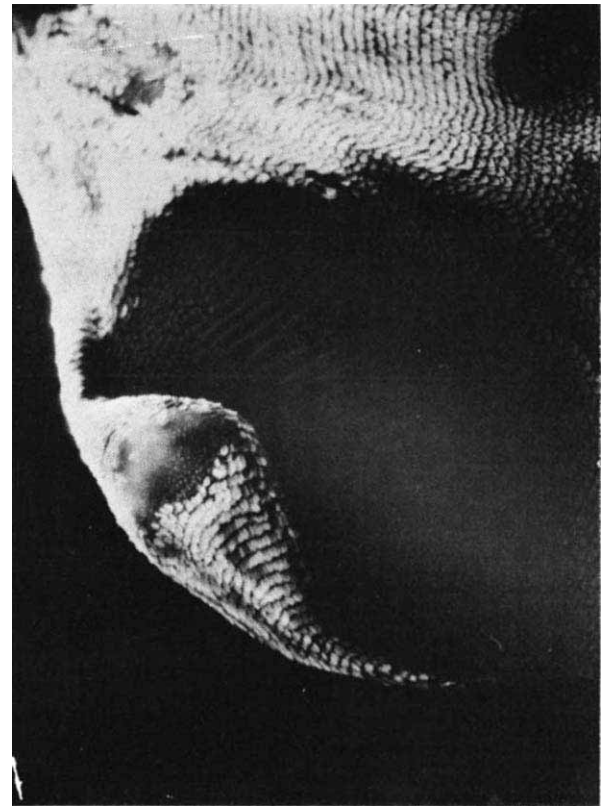
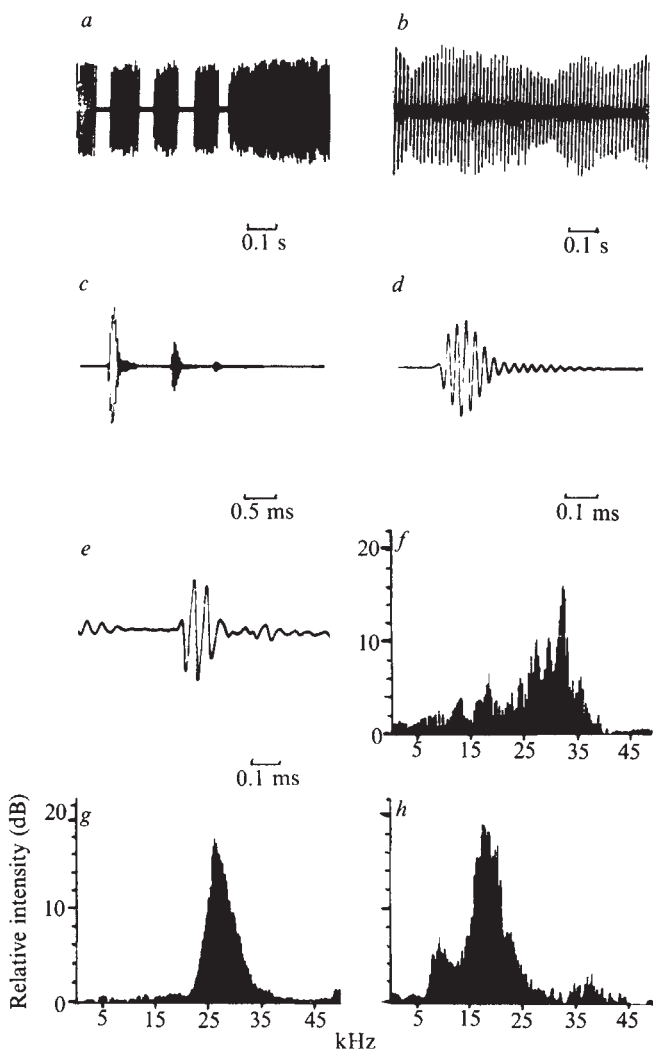


Fig. 2 Castanet of the forewing of *H. exultans* showing wear pattern and corrugated membranous area median to raised castanet.

manner, the size and number of bounces can be regulated by the force and velocity of wing closing. The corrugated membrane beneath the castanets frees the heavier tip to vibrate at its own natural vibration frequency. This situation is paralleled in certain bush crickets (*Tettigoniodea*) where the comparatively heavy 'mirror' frame portion of the sound-producing wing is free to vibrate within the membranous archidictyon^{5,6}. With the fresh wing of the moth removed, fixed at its base, and struck with a steel needle, the observed vibration frequency spectrum is close to the normal call spectrum except for the presence of side band frequencies (Fig. 1f).

Analysis of the pulse shape (Fig. 1d) reveals a wave form with no high transients expected of a percussive system of low compliance, but there is a distinctive ring with each impact involving a number of cycles before reaching maximum amplitude. This would imply that in the living system the coupling between the wings, the cavity formed by the wings and the surrounding air mass is low, that is, the sound is not dissipated rapidly after each impact. Furthermore, the slow buildup of the signal suggests that there might be a resonant air cavity loading the vibration of the wing tips. This situation is paralleled in the ensiferan grasshoppers—similar pure tone waveforms occur in the crickets and mole crickets⁷. The cavity beneath the wings is very close in its overall dimension to a sphere open at one end (Fig. 3) with a diameter approximately that of the half wavelength of the 30-kHz carrier frequency. This cavity would act as a resonant chamber set to the vibration frequency of the wings, which would explain the extended oscillation of the signal.

In contrast to *H. exultans*, *H. thyridion* spends only short periods at rest and produces its call while in flight. Each time the wings meet over the body a single pulse of sound is produced (Fig. 1e). This mechanism of sound production is under voluntary control in that the call is only emitted during the courtship flight and not in normal flight. The insect calls while performing a frenzied flight over a small area of ground; when disturbed it will fly silently some metres away and recommence its flight call. The pulse shape of the sound produced by this species indicates that each pulse is a transient with a sharp peak

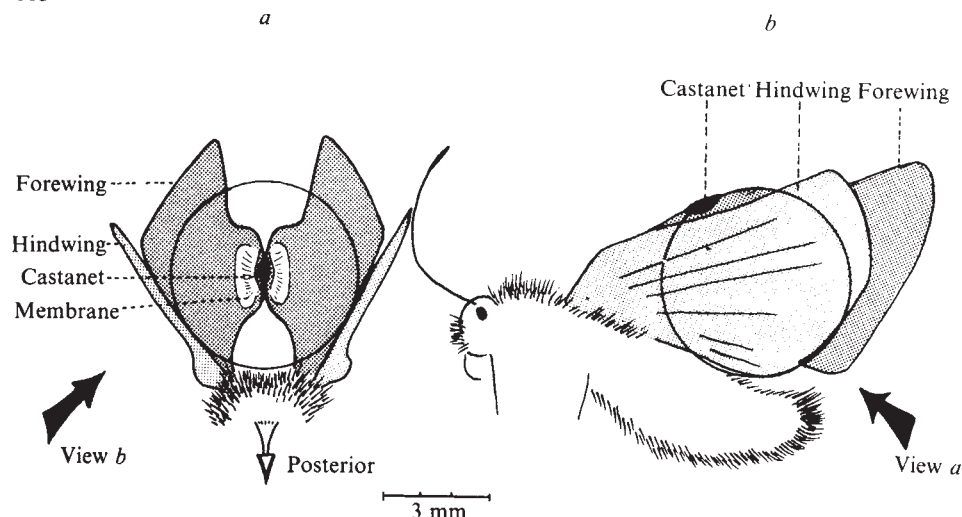


Fig. 3 Two views of the sub-wing cavity of *H. exultans*. a, View of near spherical cavity from the posterior aspect; b, lateral aspect. The circle delineates half wavelength 30 kHz.

and short duration. Nonetheless, the frequency is still highly tuned, albeit lower than that of *H. exultans* (Fig. 1h). This lower frequency is reflected in the slightly larger castanets and longer wings, which again approximate to the half wavelength of the call frequency. In this situation the sub-wing cavity is highly coupled to the outside air mass and the sound is rapidly dissipated as the wings open. A simple mechanical equivalent is seen when the thumbs of the hands are struck together with both hands linked and cupped (*H. exultans*) as compared to both the thumbs and cupped hands beating together and rapidly separating (*H. thyridion*); the former has a hollow ring whereas the latter sounds as a sharp clap.

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Urine-induced reflex ovulation in anovulatory rats may be a vomeronasal effect

EXPOSURE to urine has been shown to influence the reproductive physiology of immature and mature female rodents. It affects the onset of puberty, as measured by vaginal opening^{1,2} and by uterine growth^{3,4}, and the maintenance of pregnancy in mice^{5–7}, as well as the timing of oestrous cycles in both mice^{8,9} and rats^{10,11}. These effects seem to be elicited differentially by particular components of urine³. Although an ovulatory cycle eventually follows exposure to urine in all these examples¹², reflex ovulation (that is, ovulation occurring shortly after brief exposure to a sensory stimulus) in response to male urine, has not been demonstrated previously. Also, traditionally, behavioural, as well as gonadal effects of urine were assumed to occur through the primary olfactory system^{4,12}, rather than through one of the accessory olfactory systems, such as the vomeronasal system^{13–16}. However, a role for the vomeronasal system was recently found for male hamster mating behaviour¹⁷. Furthermore, Raisman has pointed out that the vomeronasal system is brought into relationship with neural systems that are of importance in gonadotrophic release as well as

sexual behaviour¹⁸. We demonstrate here that male urinary factors can elicit reflex ovulation in rats in the absence of coitus, mounting or removal to a novel cage. The present study is also the first to suggest that the vomeronasal system (in addition to, or instead of, the primary olfactory system) is involved in the effects of urinary chemicals on gonadal function.

In order to study chemically induced reflex ovulation, we first exposed albino female rats to continuous light (LL), a procedure which interrupts oestrous cyclicity¹⁹. The endocrine state that results from exposure to LL is characterised by persistent vaginal and behavioural oestrus, during which spontaneous ovulation occurs only infrequently and irregularly instead of regularly every four or five days²⁰. In such light-induced persistent oestrous (LLPO) rats, reflex ovulation occurs in response to coitus²¹. However, penile intromissions are not necessary for induction of reflex ovulation. Male mounts without intromission, or exposure to a novel cage previously occupied by a male, induce reflex ovulation in about 50% of LLPO rats²².

Sprague-Dawley female rats (Camm Laboratories) were first grouped (seven per cage) and then caged singly in continuous light from the time of arrival at 55 d of age. Purina rat chow and water were available *ad libitum*. The home room contained at least one adult male at all times. Females were tested between 120 and 185 d of age after showing a minimum of eight consecutive days of vaginal oestrus (as determined by daily vaginal smears). Exposure tests were conducted in home cages measuring 19 × 35 × 18 cm.

The vehicle for presenting excretory materials from males was Sanicel, a laboratory bedding made from corn cobs. Bedding soiled by Sprague-Dawley male rats (Charles River Laboratories) had been used by the males for at least one week. It was removed from the males' cages just before its use in tests. Urine was obtained on test days from several males housed on wire over collecting trays. It was pooled and then sprayed onto clean Sanicel immediately before tests. The soiled or clean Sanicel (400 ml) was placed on a clean cage floor (direct contact condition) or in a tray 3 cm beneath a wire mesh floor (no contact condition). Tests were a ½-h in duration.

Operations to occlude the canals leading to the paired vomeronasal organs (of Jacobson) were performed under light anaesthesia (Chloropent) with an electrolytic lesion maker (Grass Model LM4). A specially designed lesioning electrode was used and the indifferent electrode was placed into the anus. A lesion was made in the mucosal lining of the floor of each nasal vestibule, 4–6 mm from the surface of the external nares, approximately over the opening and anterior portion of the vomeronasal canal. Tissue damage was confined to an area about 2.5 mm long and 0.5 mm wide. Bilateral control lesions were made in the dorsolateral, mucosal lining of the nasal vestibule, with the same elec-

trode directed away from the vomeronasal pore. No lesion approached areas of olfactory epithelium. A complete description of the technique is in preparation (Mayer). These procedures were carried out 7 d before testing.

Females were autopsied 19–22 h after testing. Oviducts were placed between glass slides with a drop of saline, and examined for ova under a light microscope (100× magnification).

Groups tested, (a–h) and results, are summarised in Table 1. In our first experiment, LLPO females were given a ½ h of direct contact with bedding soiled by males (group a) or with clean bedding (group b). Ovulation occurred in 50% of the females in group a (contact with soiled bedding), but in only 15% in group b (contact with clean bedding). The soiled bedding contained ejaculatory plugs from males and faeces and urine from both males and ovariectomised females injected with oestrogen and progesterone (to bring them into heat). We hypothesised that exposure to the male urine was adequate to induce ovulation, and tested this by giving females a ½-h of contact with Sanicel that had been sprayed with either 9–10 cm³ (group c) or 20 cm³ (group d) of male urine. Although 0% ovulated when exposed to clean Sanicel sprayed with 9–10 cm³ of urine (group c), 38% ovulated when exposed to 20 cm³ of male urine (group d). Thus, the latter condition was almost as effective as exposure to soiled bedding (group a).

We then tested whether the ovulatory effect was mediated by olfaction. Groups of LLPO females were either allowed direct contact with bedding soiled by males (group e) or were separated by approximately 3 cm from the soiled bedding by a wire mesh screen on the cage floor (group f). Ovulation occurred in 49% of females in group e but in 0% of females in group f. We obtained comparable results in a replication that involved using larger cages in the home room of males maintained for mating (contact with soiled bedding: 47% of 19 ovulated; contact prevented by wire mesh floor: 10% of 20 ovulated; Fisher exact probability test $P=0.012$).

The apparent necessity for contact exposure, rather than distance exposure, to the excreta suggested that the vomeronasal organ might be involved in chemical induction of reflex ovulation. In order to test the possible role of the vomeronasal system, we occluded the canals leading to the

vomeronasal organs using electro-cautery (method described above). A control group received cautery of comparable size elsewhere in the nasal mucosa. Of females that received control lesions (group g), 54% ovulated. In contrast, ovulation occurred in only 9% of females that had received vomeronasal occlusions (group h).

We found, therefore, that brief contact with either bedding soiled by males or with male urine alone induced ovulation in about half of LLPO females within 19 h. On the other hand, when we prevented these females from contacting male excreta, by using a wire mesh barrier, ovulation did not occur. This suggested that the vomeronasal system, rather than the primary olfactory system might be involved in the reflex ovulation. We tested this hypothesis by occluding the canals leading to the vomeronasal organ. We found that when occluded females were given access to direct contact with bedding soiled by males, they did not ovulate.

We conclude that as little as a ½-h exposure to relatively large amounts of male urine is adequate to elicit reflex ovulation in LLPO rats, an effect which appears to be mediated by the vomeronasal organ. The active substance does not seem to be airborne. We are conducting further studies to determine whether the failure of LLPO rats to ovulate after vomeronasal occlusion is due to a hormonal disruption resulting from the occlusion itself or to blockage of sensory input. Preliminary findings indicate that reflex ovulation in response to mating occurs in vomeronasal-occluded LLPO rats. This supports the notion that the inability to respond to male urine, in the absence of other sensory stimuli, is related to the inability of the primary olfactory system to compensate for the loss of input to the vomeronasal system, at least during the limited time allowed. Our findings suggest that the vomeronasal system may have an important role in urine-stimulated ovulatory responses.

Two anatomically separate^{13–16,18,23} olfactory systems have now been shown to be involved in reproductive physiology. However, the relationship between these systems with respect to gonadal function has not been investigated. These new findings raise several questions: do the primary olfactory and vomeronasal systems act independently; are anatomical differences between the vomeronasal and the main olfactory systems (particularly the differences in projections within the central nervous system) related to differential effects on gonadotrophin release; are the findings that particular components of urine elicit different effects³ related to differences between vomeronasal and primary olfactory function; are the effects that do or do not depend on androgen related to different functions of the two systems?

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Table 1 Ovulatory response of LLPO rats to exposure to male excreta

Group	Environmental condition	N	No. ovulating	%	P*
a	Contact with soiled bedding	20	10	50	0.047
b	Contact with clean bedding	13	2	15	
c	Contact with 9–10 cm ³ of male urine on bedding	10	0	0	0.038
d	Contact with 20 cm ³ of male urine on bedding	13	5	38	
e	Contact with soiled bedding	29	14	49	0.001
f	Prevention of contact with soiled bedding by use of a wire mesh screen	15	0	0	
g	Control lesion and contact with soiled bedding	13	7	54	0.006
h	Vomeronasal occlusion and contact with soiled bedding	22	2	9	

Animals in groups a and e were subjected to identical experimental conditions, group a was run concurrently with groups b, c, and d, whilst group e was run concurrently with group f. If groups a and e are combined and the combined group compared with group b, the P value is 0.027.

*Probability values were assessed with the use of Fisher exact probability test.

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Lateral diffusion in plasma membrane of mouse egg is restricted after fertilisation

THE functional properties of egg membranes change rapidly at fertilisation¹. A physical basis for the earliest changes may be inferred from the observed rapid alterations in ion permeability and from the transient and prolonged changes of electrical potential^{2,3}. Subsequently, new membrane is added to the egg surface during exocytosis of cortical granules, and some surface is selectively shed in polar and midbodies^{4,5}. The physical changes occurring in the egg membrane, and the changed functional properties consequent upon them, may involve alterations of the mobility and association of lipids and proteins. Here, we present the results of studies on a general physical property, apparent lateral diffusion rate, of lipids and proteins in the membranes of unfertilised and fertilised mouse eggs. The single-cell photobleaching technique developed for measurement of diffusion in the surface membrane of cultured cells^{6–8} has been used to measure diffusion of the fluorescent dye 3,3'-diiodo-4,4'-dimethyl-6-dimethyl-2,5-bis(4-methyl-5-pyridyl)quaterphenyl iodide (diI-C18) dissolved in surface membrane lipids and of fluorescein-labelled antibody fragment bound to a spectrum of surface membrane proteins. Our results indicate that diffusion of the lipid probe and of proteins in the membrane of unfertilised eggs occurs at about the maximum rate expected for these molecules. In fertilised eggs, both markers seem to diffuse at greatly reduced rates.

Before measuring diffusion in the egg cell surface itself, the reliability of our instrument and of our data fitting was checked by determining the diffusion of fluorescein-labelled Fab fragments in glycerol solutions of various concentrations and viscosities (Table 1). A layer of solution about 80- μ m thick was formed between a slide and a cover slip. As the laser beam will bleach a cylinder running completely through such a layer⁷, recovery of fluorescence is effectively due to diffusion in two, not three dimensions. Table 1 records our values of the diffusion rate D , uncorrected for early recovery, for Fab (a cylinder of 35 Å diameter and 75 Å length) together with published values for diffusion of succinyl-concanavalin A (succinyl-con A) (radius 40 Å) in glycerol solutions of the same concentration⁷. There is generally good agreement between the two sets of experimentally determined diffusion constants, though both sets differ by an order of magnitude from values of D calculated from the Stokes-Einstein equation. This discrepancy may be due to incorrect statement of boundary conditions of the experiment. Like Jacobson *et al.*⁷, we also found that recovery of fluorescence ranged from 70–100%, with most values at about 90% of fluorescence intensity before bleaching. On re-bleaching, 100% recovery was observed.

Ova stained with the lipid-soluble dye diI-C18 appeared ring stained. The ring was, as expected¹¹, dim at the end of

the cell overlying the second metaphase spindle (here termed the pole) and bright elsewhere. Some diffuse, possibly internal, fluorescence could also be seen. We made 52 diffusion measurements on 34 unfertilised ova for diI-C18, in five separate experiments (Table 2). Recovery of fluorescence was observed in all instances. The mean value of D (D) calculated was $1.91 \times 10^{-8} \text{ cm}^2 \text{ s}^{-1}$, of the same magnitude as that reported for other cells¹⁸. Fractional recovery of fluorescence ranged from 8–49% (mean $25\% \pm 14.5 \text{ s.d.}$) of initial fluorescence. When a spot was bleached a second time, values of D were in good agreement with those calculated from the first bleach. As expected, fractional recoveries were higher in rebleached spots ranging from 50–100%, mean $68\% \pm 18.50 \text{ s.d.}$ No consistent variation in these values was seen when comparing diI-C18 diffusion at the pole, the centre or the antipode of an unfertilised egg. In unfertilised ova fixed for 1 h in 1% paraformaldehyde before photobleaching, the diffusion of diI-C18 was $0.3 \times 10^{-8} \text{ cm}^2 \text{ s}^{-1}$ (fractional recovery 20–60%) indicating that recovery of fluorescence was due to diffusion rather than cell metabolism. In addition, the shapes of the recovery curves of fixed and unfixed eggs gave no indication of a flow process as the dominant element in fluorescence recovery¹⁰.

Fertilised ova stained with diI-C18 had a uniform ring stain, again with little visible internal fluorescence. However, in only 4 out of 15 ova examined was any recovery of diI-C18 detected up to 300 s after bleaching (compared with recoveries in unfertilised eggs in the first 20–30 s after bleaching). D values for diI-C18 in eggs in which diffusion was detected were 3×10^{-9} , 7×10^{-9} , 2×10^{-10} and $3 \times 10^{-10} \text{ cm}^2 \text{ s}^{-1}$.

Fluorescein-labelled Fab fragments of xeno-antibodies (FI-Fab) reacted with the surface of unfertilised and fertilised

Table 1 Diffusion of fluorescein-labelled Fab fragments in glycerol solutions of varying viscosities

% Glycerol	D_{Fab} ($\text{cm}^2 \text{ s}^{-1}$)*	$D_{\text{succinyl-con A}}$ †
26	$12.27 \pm 0.54 \times 10^{-9}$	12×10^{-9}
55	$6.7 \pm 2.4 \times 10^{-9}$	6.4×10^{-9}
76	$2.2 \pm 0.4 \times 10^{-9}$	3.7×10^{-9}
89	$0.65 \pm 0.07 \times 10^{-9}$	1.4×10^{-9}
98	$0.5 \pm 0.15 \times 10^{-9}$	0.7×10^{-9}

Photobleaching measurements of diffusion depend on rapid production of a dark (bleached) area in an otherwise continuous fluorescent label, and the measurement of the recovery of fluorescence in the spot with time. In our experiments, a Spectra-Physics Argon ion laser, operating at a nominal power of $< 40 \text{ mW}$ at 488 nm or 514 nm was used to bleach the spot, and the same beam attenuated 10^3 to 10^4 by neutral density filters, to excite fluorescence in the spot. The fluorescein label was excited at 488 nm and measured through a green barrier filter, whereas diI-C18 was excited at 514 nm and measured through a red barrier filter. The laser beam was passed into a Leitz ortholux microscope equipped for fluorescence epi-illumination and focused on the egg with a $25 \times \text{n.a.}$ 0.68 objective. The laser beam size at the specimen was estimated at 4 μ m diameter using the method Wu *et al.*⁹. Fluorescent light emitted from the sample was passed through Leitz MPV-1 diaphragm and its intensity measured with a photomultiplier operating in a photon-counting mode (model 1140 PAR, Princeton, New Jersey); the analogue output of the photon counter drove a chart recorder. Neutral density filters were carried on a solenoid-driven arm which can be lifted away from the beam for varying lengths of time (between 0.2 and 2 s). The control box also closes the shutter to the photomultiplier tube while specimens are exposed to the full laser power. The time history of fluorescence in the bleached spot could be plotted from the recorder to trace in terms of $\log (F_t - F_0)$ against log time (F_0 = fluorescence intensity immediately after bleach) after bleaching. A graphical method for determining D from such plots has been described by Axelrod *et al.*¹⁰. The method also allows estimation of the fractional recovery of fluorescence, that is the fraction of initial (pre-bleaching) fluorescence expected in the spot at an infinitely long time after bleaching. This estimate shows how much of the label followed is immobile on the time and distance scales used in our experiments.

*Average of 4–9 determinations $\pm \text{s.d.}$ at about 26 °C.

†Published values of Jacobson *et al.*⁷.

eggs in a pattern similar to that of diI-C18: a ring stain, weak at the pole and otherwise strong around the periphery of the cell. Subjective brightness of unfertilised and fertilised eggs was similar, and this impression was confirmed when intensities of fluorescence per unit area of both sorts of eggs were measured with the photomultiplier. Sixty-eight measurements were made of diffusion of labelled Fab bound to unfertilised ova. Time of measurement ranged from 200–800 s after bleaching. Two distinct D values were observed in these measurements. A slow component of recovery was seen in all cells which were examined for times up to 500 s post-bleaching ($D = 0.16 \times 10^{-8} \text{ cm}^2 \text{ s}^{-1}$). Recoveries ranged from 24–60% after the first bleaching of a given spot and from 54–100% in re-bleached spots. In 41 of these unfertilised ova, including some which were not examined for times greater than 100 s, a rapidly diffusing population of molecules could be detected with $D = 1.45 \times 10^{-8} \text{ cm}^2 \text{ s}^{-1}$ (Table 2). This rapid component was not an artifact of background fluorescence. No recovery of FI-Fab fluorescence ($D < 10^{-11} \text{ cm}^2 \text{ s}^{-1}$) was detected in ten observations on unfertilised ova fixed for 15 min in 1% paraformaldehyde, thus precluding chemical recovery of the probe as a cause of fluorescence regeneration. Further evidence precluding chemical recovery as a cause of fluorescence regeneration was provided by bleaching the whole egg, after which no detectable recovery occurred.

A rapid component of diffusion was also seen in 9 out of 30 measurements on fertilised eggs, $D = 0.5 \times 10^{-8} \text{ cm}^2 \text{ s}^{-1}$. Values were measured at poles, centres and antipodes of eggs. Fractional recoveries were low, from 8–28%, mean $14\% \pm 7 \text{ s.d.}$ Measurements on other eggs showed no early recovery of fluorescence, and none of the 30 measurements made for as long as 500 s gave any indication of slow diffusion of Fab-labelled elements in the membrane (Table 2). These results indicate that diffusion of the bulk of both a lipid probe and of one population of labelled proteins was 10–100 times more rapid in unfertilised eggs.

Both the lipid probe and labelled proteins diffused in the unfertilised egg membrane within factor of 2–4 of the rate expected both from consideration of average membrane viscosity in mammalian and avian cells, and from previous measurements of diffusion in plasma membrane cells^{18–20}. The FI-Fab protein label was made from an anti-serum containing antibodies to many surface proteins. It reports an average D for all of these proteins. Such an average value is reasonably accurate, as lateral diffusion varies only weakly (as cube root) with molecular weight. (Our reagent gave values of $2 \times 10^{-10} \text{ cm}^2 \text{ s}^{-1}$ for lateral diffusion in mouse L-cell membranes, in good agreement with other data on this and similar cells¹⁸.) When bound to unfertilised eggs, the Fab reports two average diffusion constants for the molecules that it labels. The slower is that expected, though rarely seen, for free diffusion of a protein of 20–40 Å radius in a medium of viscosity about 1 poise¹⁸. A second, faster, value $D \sim 10^{-8} \text{ cm}^2 \text{ s}^{-1}$, is that expected for lipid diffusion. It may be that this group of labelled molecules is in fact glycolipid, but this value may also represent diffusion of loosely bound or shed surface molecules.

The proportion of all diI-C18 bound that was mobile in unfertilised eggs was lower than in some other studies. This may be due either to internalisation of some label in vesicles unable to move on the timescale of our measurements¹⁹, or to the association of the dye in some manner with immobilised proteins. The proportion of proteins labelled with FI-Fab free to move was in the range recorded for other protein and glycoprotein molecules. For both markers, re-bleaching the same spot after a primary recovery gave with few exceptions almost 100% recovery at the same rate of diffusion as recorded following primary bleach.

Diffusion of labels in fertilised eggs differed from that in

Table 2 Diffusion constants of a lipid probe and of labelled proteins in membranes from unfertilised and fertilised mouse ova

Ova	$D \times 10^{-8} \text{ cm}^2 \text{ s}^{-1} (\pm \text{s.e.})$	
	DiI-C18	FI-Fab
Unfertilised	1.91 (± 0.27) (52 observations)	0.16 (± 0.02) (49/68 observations*) 1.45 (± 0.12) (in 41/68 observations)
Fertilised	< 0.01 (in 11/15 observations) 0.2 (in 4/15 observations)	< 0.001 (in 21/30 observations) 0.5 (in 9/30 observations)

*In 19 observations, the recording of fluorescence recovery ceased before this slower recovery would have been detected.

Unfertilised mouse eggs were obtained from superovulated CFLP mice 1–4 h post-ovulation; fertilised eggs were recovered about 3 h post-fertilisation and had two polar bodies and two pronuclei. After removing cumulus cells with hyaluronidase and the zona pellucida with acid tyrode¹², eggs were exposed to dispersion of diI or FI-Fab for 15 min at 37°C. Eggs were washed by passage through several large drops of Medium PB1+10% foetal calf serum¹³, and placed individually or in small groups in wells of a microtitre test slide¹⁴ under oil, sealed by a coverslip. Eggs were examined for ring fluorescence (indicating surface labelling) before being used in diffusion measurements. Readings were taken at 25–30°C. Monovalent Fab fragments were prepared from intact rabbit anti-mouse embryo Ig (ref. 15) following the procedure of Porter *et al.*¹⁶. The Fab fragment, eluted from CM-cellulose in pH 5.5 0.01 M sodium acetate, was conjugated to fluorescein using fluorescein isothiocyanate, and the labelled fragments (FI-Fab) were separated from free fluorescein by passage over Sephadex G-25 and from overcoupled materials by re-passage through the elution from CM-cellulose. The eluted fraction (1.1 mg protein ml⁻¹) was dialysed against isotonic sodium chloride. DiI-C18 was the gift of Dr A. Waggoner, Amherst College. The dye was dissolved in absolute ethanol and this stock dispersed in PBI with protein to a final concentration of 10–20 µg ml⁻¹.

unfertilised eggs in two respects. First, the fraction of mobile label was low, varying from 0–9% for diI-C18 (compared with 8–49% in unfertilised eggs) and 0–28% for FI-Fab (compared with 24–60% in unfertilised eggs). Second, although the precise value of the diffusion constant cannot be clearly established in those eggs showing zero recovery of fluorescence during the time of measurement, it is clear that for both probes there is a substantial reduction (10–100-fold) in diffusion of both labels in most fertilised eggs. The time needed to detect any recovery of fluorescence in a bleached spot of standard size in uniform conditions depends on the diffusion constant of the label and on the mobile fraction of probe¹⁹. Our estimates of the upper limits of protein and diI-C18 diffusion in fertilised eggs (Table 2) are based on the assumption of mobility of 25–50% of the probe. Rapid recovery of a small fraction of fluorescence is found in about one-third of zygotes labelled with FI-Fab. The diffusion constant for this recovery was slightly lower than $D \sim 10^{-8} \text{ cm}^2 \text{ s}^{-1}$ recorded in some unfertilised eggs as well.

We offer four explanations for a great difference in diffusion constants when comparing unfertilised and fertilised eggs. First, the mobile proteins labelled on unfertilised eggs by our FI-Fab may be shed or rendered non-antigenic at fertilisation, and only the immobile FI-Fab-reactive antigens remain. This explanation is unlikely, as the intensity of label per unit area was within 10% when comparing unfertilised and fertilised eggs. Furthermore, this explanation does not readily apply to the lower D values measured for DiI-C18. Second, the same spectrum of proteins may be retained after fertilisation, but they may become locked into place by cytoplasmic anchors such as microfilaments or microtubules. Again, the diffusion constant for the lipid marker should not be greatly affected. Third, a change in the nature of the lipid by fusion of cortical granules might increase the viscosity of the mem-

brane thus slowing diffusion of both protein and lipid probes. It is difficult to envisage a 100-fold change in lipid viscosity arising in this way unless lipid phase changes also ensued¹⁸. Fourth, fertilisation might lead to an increased protein concentration in the membrane and the resulting solute effect on lipid viscosity might then restrict lateral diffusion of all membrane elements. Such effects of solute concentration on ordering of solvents has been discussed previously¹⁸ and effects of membrane proteins on lipid order have been noted in several reconstituted membrane systems^{21,22}.

Whatever the mechanism, constraints on lateral diffusion ought to greatly limit the possibilities for functional rearrangement and alteration of the cell surfaces of the developing zygote. This limitation could play a part in the prevention of block to polyspermy^{1,5}, in ensuring correct positioning of cells in early embryogenesis²³ and even in encoding spatial and temporal information required during cell differentiation²⁴.

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Mitotic mechanism based on intrinsic microtubule behaviour

THE molecular mechanism of mitosis in higher eukaryotic cells is largely unknown. Here, we state briefly some aspects of the mitotic mechanism that are well characterised, add information derived from our recent investigations, and offer a new model of mitotic movement based on intrinsic microtubule behaviour. We propose that two events, poleward-sliding of anti-parallel microtubules and an opposite end assembly/disassembly of all microtubules, occur constantly throughout mitosis and act coordinately to produce mitotic movement.

Our model rests on the steady-state opposite end assembly/disassembly behaviour of microtubules that we have described previously¹. Using ³H-GTP as a marker for assembly and disassembly of bovine brain microtubules under steady-state conditions *in vitro*, we have determined that the microtubule assembly/disassembly 'equilibrium' is really a steady-state sum-

mation of two different reactions which occur at opposite ends of the microtubule. *In vitro*, the assembly reaction is strongly favoured at one end, and disassembly is favoured strongly at the other end. We assume that a similar mechanism operates in the cell.

Such behaviour requires an intrinsic polarity in each microtubule. As this polar orientation of structure should be manifest along the entire length of the microtubules², the microtubules should therefore be capable of interacting with each other differently depending on whether their orientation is parallel or anti-parallel.

Briefly, we envisage a constant assembly of microtubules at the equatorial region of the spindle and at the kinetochores, coupled with a poleward migration of subunits within the microtubules as they flow towards the disassembling ends. In each half-spindle the microtubules have a parallel orientation, but inter-polar (i-p) microtubules (microtubules that originate at or near a pole and do not attach to a kinetochore, see legend to Fig. 1) from each half spindle overlap in the equatorial region in an anti-parallel fashion. A constant poleward sliding of anti-parallel microtubules past each other works against the kinetochore linkage to produce an isometric tension that is transformed to work by the uncoupling of sister chromatids in anaphase. A system of constant microtubule assembly and disassembly, of anti-parallel microtubule sliding, and of parallel microtubule linkages produces an organelle whose structural integrity is maintained by the opposition of forces in a state of dynamic tension.

Mitotic movement requires microtubules. Drugs that specifically block microtubule assembly also block mitotic movement (see ref. 3 for review). For simplicity, we will consider two classes of microtubules in the mitotic apparatus (MA), those that attach firmly at kinetochores and those that do not⁴. Most microtubules in the fully formed MA terminate (or converge) on the two peri-centriolar regions (regions that define the poles).

It is likely that microtubule assembly in the MA only initiates at microtubule-organising centres, the two peri-centriolar regions and the kinetochores⁵⁻⁷. Since microtubules have an intrinsic polarity with respect to assembly^{8,9}, it follows that microtubules approach each other from opposite centriolar regions with opposite polarity. Such an anti-polarity has been invoked in the model of mitosis based on a microtubule sliding mechanism¹⁰.

Mitotic movement at pro-metaphase evidently involves independent growth of microtubules from centriolar regions and from kinetochores, followed by the eventual convergence and interdigitation^{6,11} of kinetochore-to-pole (k-p) microtubules with parallel and anti-parallel i-p microtubules. Alternatively, a mechanism involving the growth of all microtubules from pole regions with eventual attachment of some to kinetochores could also be consistent with our model.

At metaphase, there is a 'dynamic equilibrium' of microtubules with subunits¹². The MA seems to be at rest, but there is a constant poleward flow of material as evidenced by particle¹³ and ultraviolet microbeam birefringence 'hole' migrations^{14,15}. These migration rates approximate to the anaphase rate of chromosome movement (see ref. 7).

Anaphase is characterised by separation of chromatids towards the two poles. This migration is the result of either a lengthening in the i-p microtubule system, or a shortening of the k-p system, or both^{4,16}. In those cases of anaphase MA expansion, quantitative electron microscope analysis has suggested that either anaphase separation is accompanied by sliding of anti-parallel microtubules past each other¹⁷ or that there is net i-p microtubule assembly¹⁸. Anaphase pole separation in partially lysed cells and isolated MA is dependent on ATP (refs 19, 20), however, and may not be dependent on microtubule assembly¹⁹.

We have established that microtubules have an intrinsic ability to assemble and disassemble at opposite ends at steady state¹. From this observation, we propose that the constant poleward migration of material, including microtubules, in

accord with the dynamic equilibrium concept¹³, results from constant assembly of microtubules at or near the midplane of the MA and at the kinetochore in pro-metaphase and at metaphase, with an accompanying disassembly of microtubules at the poles.

According to our model, the MA may be constructed as follows. In the first phase of mitosis, the centriolar regions act as microtubule organising centres, seeding assembly of i-p microtubules which are augmented by the addition of subunits distal to the centriolar regions. Maturation of the MA at this stage can be envisaged as resulting from an approach of its microtubules to assembly steady state. This growth may follow

release from an assembly blockage that has allowed the transition from an interphase to a mitotic microtubule system. Sliding mechanisms that are evident in anaphase may also serve to separate the two poles in prophase. Simultaneously, kinetochores assemble microtubules proximally and in this way maintain constant attachment even as k-p microtubules constantly disassemble.

If the MA is constructed in this manner, all microtubules originating in each half-spindle will have the same polarity. Anti-parallel arrangement of microtubules (for example, k-p microtubules and i-p microtubules from the opposite half spindle) leads to sliding in the direction away from the opposing pole. Therefore, chromosomes tend towards the metaphase plate at pro-metaphase (Fig. 1a).

At metaphase, i-p and k-p microtubules flow towards the poles and disassemble, while assembling either at kinetochores or at the MA equatorial region. The system is in colchicine-sensitive homeostasis²¹, indicating that microtubules must be both assembling and disassembling at this stage^{1,22}. Sliding of anti-parallel microtubules causes no net movement if it matches exactly the assembly-disassembly rate (Fig. 1b).

At anaphase, k-p microtubule assembly ceases, resulting in a net disassembly of these microtubules. Simultaneously, sister chromatid linkages are lost and the chromatids become free to migrate polewards. ATP-dependent sliding of antiparallel i-p microtubules can now drive the two poles apart. Net disassembly of microtubules is perhaps not necessary in all cases to the successful completion of mitosis, but it is the natural consequence of the microtubule steady state, following a specific anaphase assembly blockage at the kinetochore primary assembly site (Fig. 1c). In many cells, the critical mitotic movement in anaphase may be a sliding apart of anti-parallel i-p microtubules, producing a poleward tension on k-p microtubules which converge on each other near the poles, and attach to i-p microtubules through parallel linkages. These enable microtubules to converge without attaching to a specific polar element, and allow for the coordinated migration of the chromatids in anaphase, a phenomenon which is commonly observed. As the process proceeds through telophase, disassembly proceeds from the centriole towards the daughter cell interface region, producing a midbody.

We believe that this model accounts for all the movements associated with the typical higher eukaryotic mitosis. It fits with our understanding of the mechanism of colchicine sensitivity²², the steady-state opposite end assembly and disassembly of microtubules¹, the existence of ATP-dependent sliding mechanisms between microtubules^{22,24}, and the observation of linkages between microtubules lying in parallel register in the MA (refs 17, 25).

Most of the critical cytological observations on mitosis are also consistent with this model. These include the repetitive pole-to-pole migrations of unpaired X chromosomes in grasshopper spermatocytes²⁶, which we interpret as being due to the repeated linkage of unpaired k-p microtubules with the near (anti-parallel) half-spindle i-p microtubule system; the interdependent movement of chromosomes in anaphase²⁷, which we interpret as resulting from linkage between parallel microtubules; while the manner in which ultraviolet microbeam destruction of one kinetochore fibre causes a bivalent to jump towards the other pole at metaphase²⁸ is possibly caused by the isometric poleward tension produced by anti-parallel microtubule sliding at metaphase. Also relevant are the many reports of chromosome oscillations at the metaphase plate¹⁷. Assuming the onset of assembly blockage at the kinetochore to be variable relative to the onset of anaphase movement, the literature confirms our expectation that k-p distances will remain constant as pole-to-pole distances increase (late blockage)¹⁷, or that they will decrease in anaphase (blockage in concert with the onset of anaphase)¹⁷, or that the entire MA will shorten at metaphase and increase in length again at anaphase (early blockage)²⁹.

An actin-myosin system has been postulated to exist in the MA, based on direct observation³⁰, and on observations from

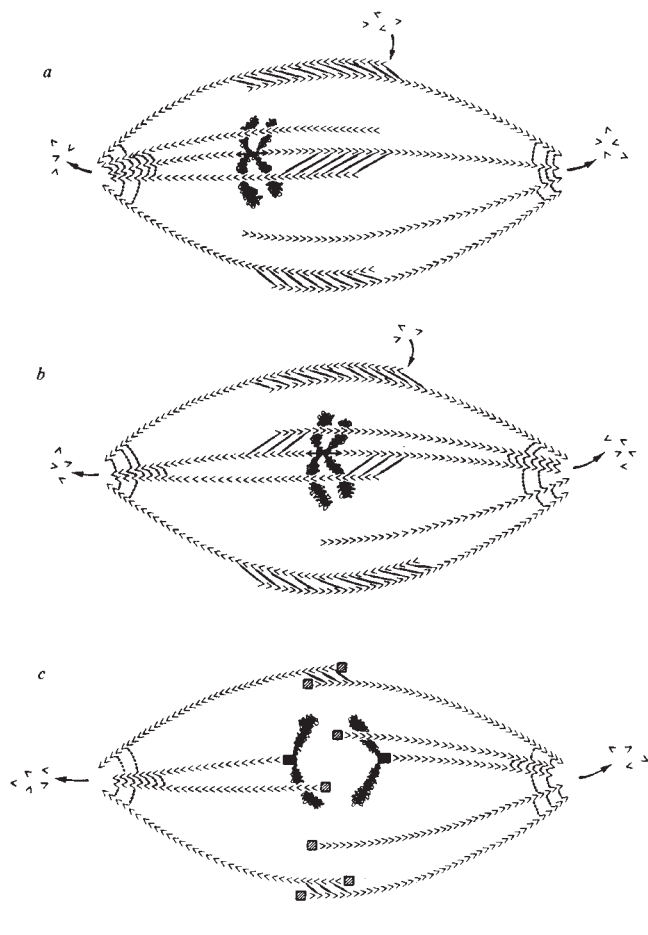


Fig. 1 A schematic representation of the proposed mitotic mechanism. The spindle region of a typical higher eukaryotic MA is depicted. Arrow lines indicate microtubules. The arrows represent the direction of dimer flow in the microtubules created by steady-state assembly-disassembly dynamics and by the sliding of anti-parallel microtubules past each other. Microtubules are parallel or anti-parallel depending on whether their arrows point towards the same or opposite poles respectively. Those microtubules that attach to chromosomes are kinetochore-to-pole (k-p) microtubules, all others are inter-polar (i-p) (microtubules of various lengths, which originate at or near a pole, and traverse the spindle). The kinetochore, the organelle by which k-p microtubules attach to chromosomes, is represented by a line through the metacentric chromosome constriction. *a*, In pro-metaphase there is a re-establishment of microtubule assembly, following a transitional assembly blockage that permits the dismantling of the interphase microtubule network, and the establishment of organising centres for the MA. Anti-parallel microtubule sliding at this stage causes chromosomes to migrate towards the metaphase plate. *b*, At metaphase microtubules are at steady state. The MA structure is maintained by the internal tension of antiparallel i-p microtubule sliding that pulls on sister chromatid linkages. *c*, Anaphase is characterised by the cleavage of sister chromatid links, and a simultaneous blockage of microtubule assembly at the kinetochore (solid blocks) and possibly, but not necessarily, at the equatorial region (hatched blocks), while i-p microtubule sliding continues. ●—●, Parallel microtubule linkages; |, anti-parallel sliding linkages between microtubules.

fluorescent staining^{31,32}, but its function in mitosis remains a matter of controversy. Anti-actin and anti-myosin antibody do not interfere with ATP-dependent anaphase movement in partially lysed cells⁷, in isolated MA (ref. 20), or following microinjection into whole cells (anti-myosin antibody)³³, even when the same antibody effectively blocks cytokinesis^{20,33}. In contrast, however, antibody to the tubulin-associated protein, dynein, does seem to block anaphase progression²⁰. We offer here a mechanism in which various aspects of microtubule behaviour interplay to produce an inevitable orderly separation of chromosomes. In this model, there is no need to invoke an actin-myosin system for the mitotic process.

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Mitogenic effect of α -mannosidase on lymphocytes

MITOGENS induce blastogenesis and mitosis in lymphocytes after reacting with specific receptors on the cell surface. In contrast to antigen receptors, the receptors for mitogens may be common to large fractions of the lymphocyte population. For example, the lectin concanavalin A (con A) causes mitosis in most T cells. The specificity of the receptors is believed to depend on constituent carbohydrate residues, and the effect of mitogens can be modified strongly by enzymes which govern the removal of carbohydrates

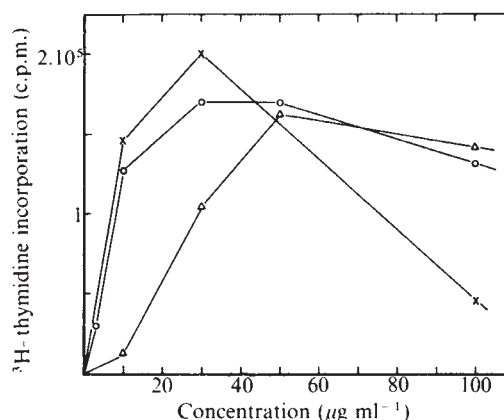


Fig. 1 DNA synthesis measured by incorporated ^3H -thymidine in lymphocyte cultures after 72 h of incubation with various concentrations of α -mannosidase I (○), α -mannosidase II (Δ) and con A (×). Activity in controls was about 500 c.p.m. Each point represents the mean of at least three cultures. Lymphocyte suspensions (including monocytes) were prepared from fresh, human, heparinised blood by centrifugation on a Ficoll-Triosil gradient. After washing three times, cells were resuspended to a concentration of about 1.5×10^6 per ml in medium RPMI 1640 with 10% human AB serum and antibiotics. Cells were incubated in 0.1-ml samples with α -mannosidase or con A (Sigma) in microassay trays (Linbro) at 37°C , 5% CO_2 and high humidity. Methyl- ^3H -thymidine ($1.25 \mu\text{Ci}$ per sample) was added 3.5 h before collection of cells for analysis.

from the cell surface¹. Thus these enzymes resemble mitogens in that there are specific reacting sites for them on the cell surface. We report here an enzyme which seems to be mitogenic in itself: α -mannosidase produces blastogenesis and mitosis in lymphocyte cultures with an efficiency comparable with that of con A. As α -mannosidase is found in various tissues and cells, including lymphocytes, as well as in blood plasma²⁻⁴, our observation may have interesting immunological implications. Another enzyme, galactose oxidase, has been reported to be mitogenic although less strongly than our enzyme⁵.

α -Mannosidase was extracted from the bean of *Phaseolus vulgaris* and purified to homogeneity by a specific antibody column technique as before⁶. Two forms of the enzyme (I and II) were obtained, both being glycoproteins consisting of two noncovalently bound subunits each of molecular weight about 110,000, but with significant differences in carbohydrate and amino acid composition and with $p\text{I}$ values of 5.1 and 6.1 respectively⁷.

Lymphocyte cultures were incubated in standard conditions with various concentrations of α -mannosidase and con A and withdrawn for analysis at appropriate times. The

Fig. 2 DNA synthesis in lymphocyte cultures as a function of time of incubation with α -mannosidase I ($30 \mu\text{g ml}^{-1}$, ○) and con A ($30 \mu\text{g ml}^{-1}$, ×). Experimental conditions were as for Fig. 1.

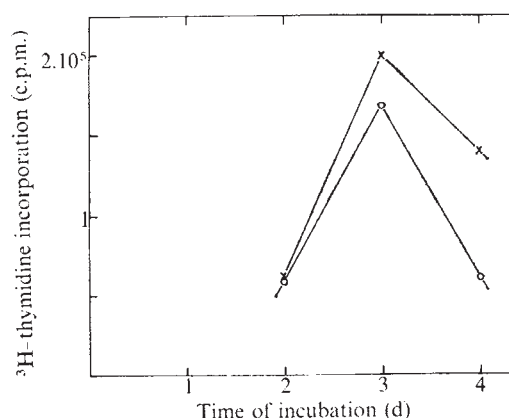


Table 1 DNA synthesis in lymphocyte cultures after 72 h of incubation with various stimulants in three experiments with cells from different donors

Stimulant	Source	Optimum conc. ($\mu\text{g ml}^{-1}$)	^3H -thymidine incorporation (c.p.m. $\times 10^{-4}$)		
			1	2†	3
α -Mannosidase I	<i>P. vulgaris</i>	30	17	14	7.5
α -Mannosidase II	<i>P. vulgaris</i>	50		13	
α -Mannosidase-rich liver extract	Human liver	$\geq 150^*$			3
Con A	Jack bean (Sigma)	30	20	6	12.5
Control	—	—	0.05	0.05	0.05

Experimental conditions were as for Fig. 1. α -Mannosidase was extracted from human liver by ammonium sulphate fractionation, ion exchange chromatography, gel filtration, isoelectric focusing and analytical ultracentrifugation on a 5–20 sucrose gradient.

*Total concentration of protein in a liver extract with a specific enzyme activity at pH = 4.5 one tenth of that of α -mannosidase I.

†Serum concentration in this experiment was 20%.

rate of DNA synthesis was measured by incorporation of ^3H -thymidine. Blast formation was monitored by volume spectroscopy using an electronic transducer of the Coulter type⁸.

Figures 1 and 2 show that in terms of ^3H -thymidine incorporation the mitotic response to α -mannosidase I was approximately the same as to con A with respect to both mitogen concentration dependence (Fig. 1) and the time kinetics of the reaction (Fig. 2). But the mitotic effect was not reduced at higher concentrations as it is with con A, apparently because of cytotoxicity⁹. Furthermore, the DNA synthetic cycle seemed to be completed earlier with α -mannosidase (Fig. 2). The weight concentration of α -mannosidase I giving maximum effect was the same as for con A— $30 \mu\text{g ml}^{-1}$. Because the molecular weight of α -mannosidase is 220,000 compared with about 110,000 for con A¹⁰, α -mannosidase I is about twice as efficient as con A on a molecular basis. Essentially similar results were obtained in three separate experiments (Table 1).

α -Mannosidase II had a mitogenic effect per molecule, approximately half of that of α -mannosidase I, whereas the maximum level of DNA synthesis was the same (Fig. 1). Like con A, α -mannosidase had a pronounced agglutinating effect on the cells, observable after a few hours of incubation. Blast transformation, however, does not depend on agglutination.

The mitotic effect of α -mannosidase was not reduced by increased concentrations of serum (up to 20%, Table 1). Thus the effect is not blocked by serum proteins, as seems to be the case for con A.

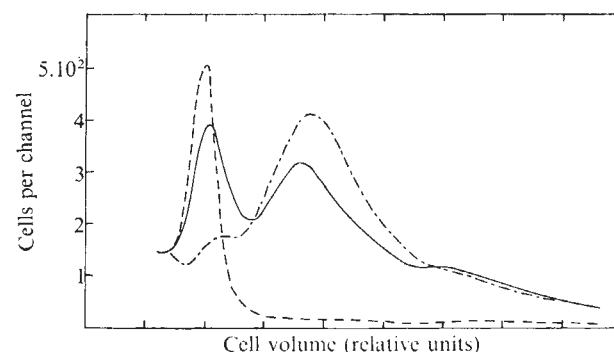
^3H -thymidine assay gives only an average value for the entire cell culture, with no direct indication of mitosis. In contrast volume spectroscopy measures individual cells and yields volume distributions from which the fraction of cells actually growing and dividing can be determined. The volume distributions in Fig. 3 are evidence that α -mannosidase and con A induced growth and division of cells. The fraction of cells that had undergone blast transformation after 4 d was significantly larger with α -mannosidase, about 85% compared with about 66% with con A. This difference

is consistent with the results in Fig. 2, which indicate that blast transformation induced by α -mannosidase is completed faster than that induced by con A. It also seems that α -mannosidase stimulates a larger fraction of the lymphocytes than does con A. The large percentage of blasts implies that α -mannosidase is primarily a T-cell mitogen, although it cannot be excluded that B cells are also stimulated.

α -Mannosidase from *Phaseolus vulgaris* was highly homogeneous, as judged by several established criteria, including SDS electrophoresis and analytical ultracentrifugation⁷. The mitogenic leucoagglutinin from *P. vulgaris*, phytohaemagglutinin, had completely different migration characteristics, both by conventional disc electrophoresis and in the presence of SDS. The low concentration of α -mannosidase needed for stimulation makes it unlikely that the mitotic effect is due to impurities.

The discovery that an enzyme is an efficient mitogen raised the question of whether it has immunological significance *in vivo*. It was not obvious, however, that the mammalian form was mitogenic. To test this, α -mannosidase was extracted and purified from human liver. As Table 1 shows, this extract had a mitotic effect per unit enzyme activity of the same order of magnitude as α -mannosidase I. Thus ^3H -thymidine uptake was much larger than can reasonably be ascribed to a possible antigenic effect of other proteins present in the liver extract as impurities. The extract also caused agglutination of the lymphocytes to approximately the same extent as did α -mannosidase I and con A. A similar extract from another liver gave corresponding results (Table 2). The percentage of enlarged

Fig. 3 Distribution of cell volumes in lymphocyte cultures after 4 d of incubation with α -mannosidase I ($30 \mu\text{g ml}^{-1}$, ---) and con A ($30 \mu\text{g ml}^{-1}$, —). The curves are smoothed histograms comprising 255 channels along the abscissa. The volume of unstimulated controls (---) was constant throughout the experiment. The peak at about twice the volume of the control represents cells divided as a result of mitogenic stimulation, whereas the shoulder at higher volumes is primarily cells in G_2 and mitosis. Cell aggregates were resolved by incubation with 20 mM EDTA and 50 mM α -methyl-mannoside at 30°C , 5% CO_2 for 3 h before collection.

**Table 2** DNA synthesis and blast formation in lymphocyte cultures after 72 h of incubation with liver extracts compared to response with optimal concentration of phytohaemagglutinin

Stimulant	^3H -th. incorp. (rel. units)	Large cells (%)
α -Mannosidase-rich liver extract	10	14
α -Mannosidase-free liver extract	0.7	14
PHA-M (2%)	100	53
Control	0.7	9

Experimental conditions were as for Fig. 1 and Table 1. The control value of large cells represents mainly monocytes. PHA-M, phytohaemagglutinin (Gibco).

cells produced by this extract corresponded with its mitogenic effect. A liver extract with no detectable α -mannosidase activity gave no response (Table 2), suggesting that the mitogenicity is associated with the enzyme. We conclude that a liver extract rich in α -mannosidase is strongly mitogenic. The purity of the liver extracts, however, was not so high that the effect can be ascribed unequivocally to the enzyme.

The most obvious interpretation of our results is that α -mannosidase acts as a mitogen in a manner similar to that of mitogenic lectins, that is after binding to specific sites on the cell surface. But it is possible that the enzyme activates potential mitogenic serum factors, either by modifying glycoproteins in the serum or by altering carbohydrate structures on the cell surface so as to facilitate the binding of such factors.

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Possible reversion of malignancy *in vivo*

CULTURES of transformed cells in which the transformed state has become a hereditary property can produce variants *in vitro* which represent a reversal of transformation. The characteristics of these variants include contact inhibition, low cloning efficiency in liquid medium and inability to form colonies in soft agar or tumours *in vivo*. They are thus less tumorigenic than the parental transformed cells¹. We report here that such variants can be obtained *in vivo* by serial transplantation of tumour cells in allogeneic mice.

Rabinowitz and Sachs² have suggested a model for the genetic basis of malignancy. They have proposed that expression and suppression of transformed properties depend on the balance between factors responsible for expression (E) and suppression (S), and that these factors can be located on chromosomes. The variants have a much higher chromosome number than the transformed cells³, which suggests that an increase in the appropriate chromosomes can lead to reversion. We have sought evidence for this model *in vivo*.

We used the murine lymphoma line L5178Y which is stable by syngeneic passage in BALB/c mice, with a diploid chromosome number of 39 ± 1 . When serially allotransplanted for up to eight passages in CD1 mice (Charles River Laboratories) the cells lost their tumorigenicity (Table 1). The reverted cells produced in CD1 mice were not tumorigenic when transplanted into the original host BALB/c mice.

The reversion state was associated with an increase in chromosome number from diploid to subtetraploid or tetraploid between the first and fifth transplantation and with a decrease to diploid or subdiploid between the sixth and eighth transplantation (Fig. 1). Whereas transformed cells had the expected diploid chromosome number of 39 ± 1 , reverted cells changed to 74–84 in the first transplantations and to 25–39 in later transplantations. The maximum percentage of cells with these new

Table 1 Tumour formation in CD1 mice

Serial allotransplantation	Tumour incidence*	Average time to death (d)
1	10/10	10
2	10/10	10
3	9/10	10
4	8/10	10
5	7/10	10
6	7/10	12
7	5/10	12
8	3/10	12
9	0/12	—

50×10^6 cells per mouse were inoculated intraperitoneally. Each transplantation was made with cells from a pool of ascitic fluid collected from mice with tumour from anterior transplantation on the 9th to 11th day.

*No. of animals with tumour/no. of animals inoculated.

karyotypes were 25% and 15%, respectively. (In normal and transformed cells we found less than 1% in both cases).

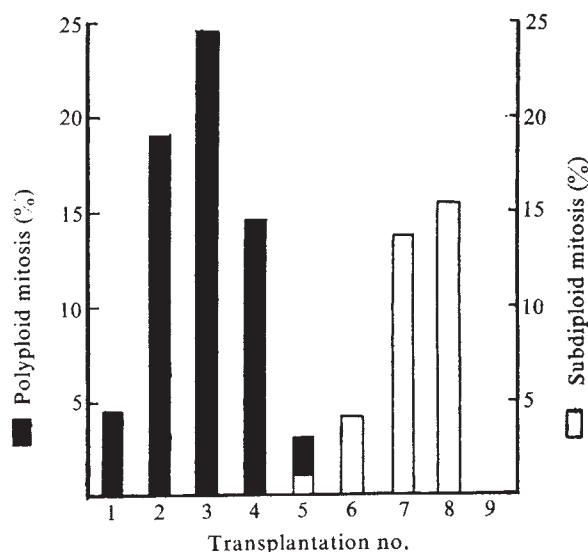
Polyploidy, though common in plants, is rare in animals. However, mammalian cells grown in tissue culture frequently become polyploid. This must be the cause of the phenotypic changes in such cells.

The stability of the reverted cells can be explained by the balance between chromosomal factors E and S that determine the expression and suppression of transformed properties³.

Our data provide evidence for the model in which the expression and suppression of transformation depend on the balance between chromosomes with E and S. Thus, transformed cells were associated with an increased number of chromosomes with E which established an excess of E over S. Variant cells established an excess of S over E. The delay in expression of transformation after animals are treated with carcinogen may correspond to the time required for the increase in chromosomes with E (ref. 4). This phenomenon must be similar to the gradual transformation found in certain somatic cells when they are established and cultured *in vitro*⁵.

The environment provided by the allogeneic host must be important in the reversal of tumour cells. But the situation is different from immunological rejection because at first all mice accepted the tumour cells. The percentage accepting tumour

Fig. 1 Solid bars represent the percentage of polyploid mitosis in cells collected from ascitic fluid of CD1 mice. In the sixth to eighth transplantation all abnormal mitotic cells were subdiploid. The polyploidy appearing in the first days is related to the initiation of reversion of malignancy and the subdiploidy to the establishment of fewer chromosomes with factor E, responsible for expression of transformed cells.



cells declined after the second transplantation until the ninth transplantation when the transplanted cells were no longer tumorigenic. Furthermore, in a real immunological rejection those animals which do not accept the tumour when given a subtumorigenic quantity of cells remain permanently immunised and do not accept the tumour in further challenges.

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Myeloid leukaemia in X-ray irradiated CBA mice

ABOUT two-thirds of the excess leukaemia in adult Japanese survivors of the atomic bombs is myeloid in type (acute plus chronic)¹. Nevertheless, most of the extensive experimental work on leukaemia induction by ionising radiation in the past 25 yr has been concerned with the thymic leukaemia of the laboratory mouse², a disease which may have no human counterpart. So far, only one series of systematic experiments has been reported on myeloid leukaemia in irradiated mice^{3,4}, and this concerned RF mice, a strain which has this disease in the relatively high frequency of 2–6% in unirradiated controls. Single brief whole-body X-ray exposures have now proved to be effective in inducing myeloid leukaemia in male CBA/H mice, provided that the radiation dose is not too great. This possibly provides an important model for quantitative investigation of carcinogenic mechanisms and for use in the development of chemotherapy for the analogous human disease.

The minimal criteria for post-mortem diagnosis were complete, or almost complete, replacement of sternal bone marrow by abnormal myeloid cells and marked diffuse and focal infiltration of the liver by similar cells. There was usually also an extra-medullary infiltration of sternal muscle, a markedly enlarged spleen consisting very largely of abnormal myeloid cells, and infiltration of the kidney. In subsequent experiments with the same strain of mouse and similar radiation doses, the terminal white blood cell count was in the range 10^4 – 10^5 mm⁻³ and most of the white cells were abnormal myeloid cells of various degrees of immaturity. Inoculation of spleen cells into syngeneic recipients led to the deaths of the recipients from myeloid leukaemia in every case in which this was tried.

Major involvement of the bone marrow by malignant lymphoreticular cells was quite uncommon after 75–300 rad, but such cases were about as frequent as myeloid leukaemia after 450–600 rad. Thymic leukaemia was not seen, although high frequencies can occur in irradiated CBA/H mice in the appropriate experimental conditions⁵.

The dose-response relationship was highly curvilinear and the optimum X-ray dose was in the region 150–300 rad (Fig. 1). A threefold increase in dose above this decreased the frequency of induced myeloid leukaemia by an order of magnitude. Figure 2 shows the distribution of times to death for cases of myeloid leukaemia and that the reduction in frequency with

larger doses was not simply the consequence of failure of the most heavily irradiated mice to survive. (Full details of this work will be reported elsewhere.)

The diagnosis of myeloid leukaemia was usually straightforward. Post-mortem autolysis sometimes made cytological diagnosis uncertain: 'possible but not definite' cases, about 5% of the total, are excluded from Figs 1 and 2.

Myeloid leukaemia was not found in 190 unirradiated control CBA/H males which were kept with their irradiated littermates in the same cages throughout their lifespan, apart from the day on which X-ray exposure was made. It has not been seen in further controls in continuing experiments. Thus, in order to induce murine myeloid leukaemia by radiation it is not obligatory to use a strain naturally prone to it.

The reduction in leukaemia frequency at larger doses of radiation was substantial and statistically significant. This is also true in man⁶. The simplest explanation for such a dose-dependent reduction is the interaction of two concomitant effects of radiation, transformation of normal cells into leukaemia mother cells and cellular inactivation. Inactivation, the loss of the ability to undergo sequential cell division (with or without death of the cell) has been postulated for many years as a factor interfering with the development of overt malignant disease in irradiated subjects (see for example, ref. 7).

The observations on myeloid leukaemia in RF mice given single brief X-ray exposures could be fitted quantitatively by this hypothesis⁸ but of themselves were not an adequate test of it. There was no clear reduction in leukaemia frequency with increasing dose and, in order to assess the true yield of myeloid leukaemia, correction for competing causes of death was required³, because thymic leukaemia was also induced at similar frequencies and at similar or even younger ages^{3,4}. In CBA mice, however, myeloid leukaemia frequency was positively reduced by increasing dose (Fig. 1) and there was no other major, radiation dose-dependent, competing cause of death during the period when most myeloid leukaemia was seen (Fig. 2).

The hypothesis of competition between malignant transformation and cellular inactivation seemed to be widely applicable to human data on radiation induction of cancer¹, but no direct test of the hypothesis can be made in man. Curve fitting of experimental data is similarly inadequate. Continuing observations show that myeloid leukaemia frequency in CBA mice given 300 rad is markedly reduced (or, alternatively, postponed) by a further dose of 600 rad given 1, 4, 16 or 32 weeks later. It is difficult to imagine any other explanation for this than inactivation of the developing leukaemia cells by the second dose. A corollary is that the leukaemic transformation by 300 rad is a rare event in cellular terms and that the initial rate of cell division of transformed cells is low, so that normal haematopoietic stem cells greatly outnumbered the transformed cells at the time the 600 rad was given. Animals would not otherwise

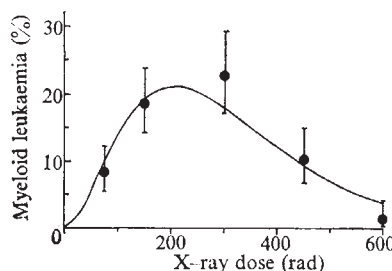


Fig. 1 Lifetime frequency of myeloid leukaemia in male CBA mice (definite diagnoses only) and brief whole-body X-ray dose. Mean values $\pm 80\%$ Poisson confidence limits. Each mean is the pooled value for similar numbers of mice irradiated at 550, 54 or 3.7 rad min⁻¹. The curve is the best fit to the expression aD^2e^{-bD} ($P = 0.25$) where D = dose in rad, $a = 3.7 \pm 0.8 \cdot 10^{-5}$ rad⁻² and $b = 9.7 \pm 0.7 \cdot 10^{-3}$ rad⁻¹. The expression aDe^{-bD} does not fit ($P = 0.006$). The number of irradiated mice per point was 119–156.

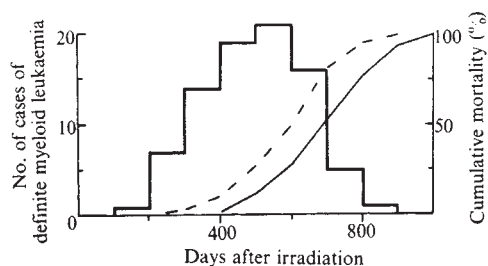


Fig. 2 Distribution of deaths with myeloid leukaemia (definite diagnoses only) and cumulative mortality (excluding myeloid and all other leukaemias) against time after irradiation (d). The histogram gives the number of myeloid leukaemia deaths (all doses combined) in successive 100-d periods. ----, Cumulative mortality curve for 600 rad; —, unirradiated controls. The cumulative mortality curves for the other X-ray doses lay between these two. Most of the cases of leukaemia occurred before non-leukaemic mortality became appreciable.

have survived 300+600 rad without also developing overt leukaemia in the frequency expected after 300 rad alone. The rarity of malignant transformation *in vivo* as compared with other cellular effects of ionising radiation seems clear¹.

Analysis in terms of the hypothesis under discussion suggested that induction of myeloid leukaemia by γ or X rays in RF mice depended approximately on the square of the dose⁸. This seems also to be the case in CBA mice (Fig. 1). The value of b in the equation fitted to the data (Fig. 1) lies within the accepted range for murine haematopoietic stem cells⁹, as it should do if the hypothesis of competition between induction and inactivation is valid, and if myeloid leukaemia is initiated by transformation of such stem cells.

An increase in whole-body X-ray dose of the mouse from 150 to 300, and even more so to 600 rad, decreases a range of immunological responses¹⁰. As the same change in dose decreases the occurrence of myeloid leukaemia quite markedly, decrease in immunological responsiveness would seem not to be a factor favouring the induction of myeloid leukaemia or, by extension, of other kinds of malignant disease. Simple hypotheses involving viral release or induction would also predict an increasing effect with increasing radiation dose but clearly some allowance should be made for the numbers of host cells involved.

After years of failures, the experimental conditions which allowed the induction of myeloid leukaemia in CBA mice proved to be unexpectedly simple. The mistaken assumption was that induction would be favoured by increasing the degree of damage in the target tissue, that is, by using large radiation doses. This, and the narrow range of dose permitting the easy demonstration of myeloid leukaemia induction (Fig. 1), may have implications for the testing of chemical compounds for carcinogenicity, provided, of course, that ionising radiation is not a special case.

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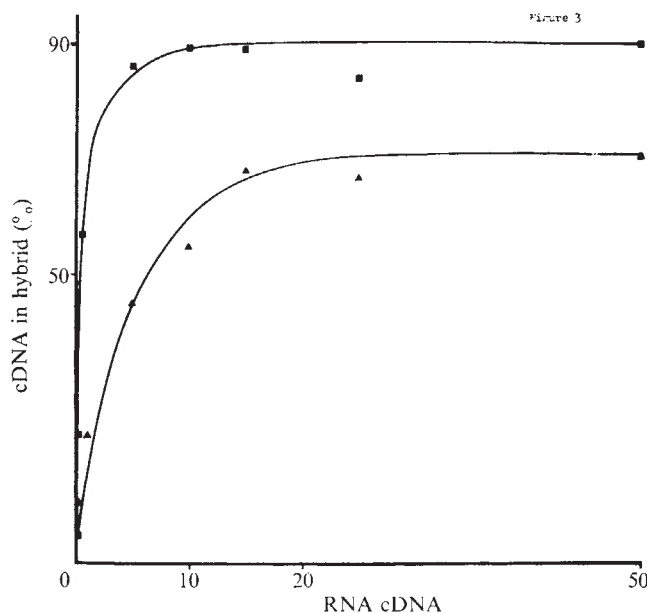
Are spleen focus-forming virus sequences related to xenotropic viruses and expressed specifically in normal erythroid cells?

DEFECTIVE type C RNA tumour viruses which are genetic recombinants between ecotropic and xenotropic viruses have been described and suggested to be the real transforming agents during the course of viral-induced lymphatic leukaemia^{1,2}. The spleen focus-forming virus (SFFV-F) component of the Friend virus (FV) complex is a defective virus and is the erythroid-specific component of the complex which stimulates erythropoiesis in the mouse and is also involved in the relatively rare event of erythroid transformation^{3,4}. In order to examine the role of the SFFV component during erythroblastosis and subsequent transformation of erythroid cells, we have synthesised a SFFV-specific probe using FV released from F4-6, a transformed erythroid cell line^{5,6}. We report here that, using this probe, no xenotropic sequences are present in the SFFV-specific cDNA and that there is a low level of transcription of part of the SFFV sequences but not specifically during normal erythroid differentiation. Our results thus show that if SFFV is a recombinant virus, the xenotropic sequences are only present in the lymphatic leukaemia virus (LLV)-related common sequences of the FV genome. Furthermore, our experiments do not support a hypothesis whereby the specific action of SFFV can be explained by transcription of the SFFV-specific genome during normal erythroid differentiation.

The LLV-F helper virus and the SFFV-F components of the FV complex can be cloned in tissue culture cells^{7–9}. The cloned virus in SFFV-infected cells is defective and the cells have to be superinfected by helper virus to rescue SFFV as part of the FV complex^{7,8,10}. Troxler *et al.*⁸ synthesised SFFV-specific cDNA using FV rescued from infected non-producer fibroblast lines^{7,8} and showed that the SFFV-specific cDNA is 50% homologous to the RNA of xenotropic murine leukaemia virus (MLV-X). Furthermore, their results suggest that SFFV is a recombinant between ecotropic MLV and the *env* gene region of xenotropic type C virus¹¹.

This seems highly significant since similar recombinant viruses have been described and suggested to be the real

Fig. 1 Titration of F4-6 60S RNA and LLV-F 70S RNA with FV cDNA. FV cDNA (1 ng) was titrated with increasing amounts of F4-6 60S RNA (■) and LLV-70S RNA (▲). RNA isolation, FV cDNA preparation and hybridisations were carried out as described in Table 1.



transforming agents during the course of virally induced lymphatic leukaemia^{1,2,12}. Considerable doubt still remains, however, as to whether the passage of SFFV from the erythroid target cells to fibroblasts results in selection of a recombinant variant of SFFV (refs 7, 8).

We have isolated the LLV-F helper virus from the original complex released by F4-6 cells by two successive end-point dilutions of the viral complex on SC-1 cells^{9,13}. The helper virus of cell line 643/22 isolated in this way induces lymphatic leukaemia in newborn mice, but the supernatants of these leukaemic spleen cells do not induce any spleen foci nor do they induce erythroleukaemia in more than 100 mice which we have inoculated with this virus. To ensure that the SFFV is not just a minor component of the FV complex which we have used, we determined the relative proportions of biologically active LLV-F and SFFV in the FV complex. The Friend virus complex released by F4-6 cells was used to infect SC-1 cells. The supernatant from 10^6 FV-6 cells was added to 10^6 SC-1 cells. The infected SC-1 cells were cloned in microtitre wells 45 min after exposure, and 29 single-cell clones, as determined by visual inspection, were isolated and tested for the presence of reverse transcriptase (RT) in the supernatant. Twenty-three of the single-cell clones were RT positive and can thus be assumed to have been infected by at least LLV-F. To our surprise, virus from all 23 virus-positive SC-1 clones was also capable of forming spleen foci in DBA/2J or BALB/c mice. In addition, 5 of the 6 virus negative SC-1 clones were shown to release SFFV on superinfection with LLV-F of cell line 643/22. When the Poisson distribution is used to calculate the 'hit number' the data indicate that the LLV titre is 1×10^6 per 10^6 F4-6 cells and the SFFV 3×10^6 per 10^6 F4-6 cells. This estimate thus shows that SFFV-F is in excess over LLV-F and the biological titre of both components is very high in our FV complex. The same supernatant of the F4-6 cells which was used for the single-cell infections, was injected directly into the tail vein of DBA/2J mice and yielded only 1.2×10^3 spleen foci from the supernatant of 10^6 F4-6 cells. This indicates that the detectability of spleen focus-forming activity in the FV complex released by transformed erythroid cells is 10^{-3} of the real biological titre. A discrepancy of 10^{-1} – 10^{-2} has also been shown previously^{7,8}.

We have also checked three of the SFFV-positive and RT-positive SC-1 cell lines for their viral subunit structure. All three viral isolates contain not only the high molecular weight 35S RNA (3.3×10^6) but also 32S RNA of 2.6×10^6 size (data not shown). This shows that the SFFV/RT positive clones used for estimation of the relative amounts of SFFV and LLV in the F4-6 complex, did in fact, still release SFFV and LLV-F and not just a new helper-independent SFFV.

Virus from F4-6 cells not exposed to DMSO was used in order to avoid complications caused by induction of an SFFV complex with an altered host range which might involve the induction of endogenous ecotropic and perhaps also xenotropic virus^{9,14}. Complementary cDNA was synthesised by using lysed virions (ref. 5, 9 and I.B.P. and W.O., unpublished results). The cDNA is not full length but in pieces of 10–20S size as indicated by the mobility on formamide gels (data not shown). Nevertheless, hybridisation analysis shows that the cDNA is uniformly representative of the whole FV genome, as 70% 32 P-FV 60S RNA is protected at a ratio of cDNA–RNA of 1:1. Our FV cDNA is thus very similar in this respect to that used by others⁹. The cDNA of the F4-6 FV-complex was then annealed to 70S RNA of the LLV-F of cell line 643/22 and 60S RNA from F4-6 virus as a control (Fig. 1). The differences in plateau value show that the removal of LLV-F sequences from the total FV cDNA probe by hybridisation to 643/22 70S RNA is possible. The cDNA remaining unhybridised in this experiment (which should contain SFFV-specific complementary sequences) was therefore isolated by hydroxyapatite chromatography as shown in Fig. 2. It was necessary to perform two cycles of hybridisation to 643/22 70S RNA to remove all sequences complementary to LLV-F. The SFFV-enriched cDNA was further purified by hybridisa-

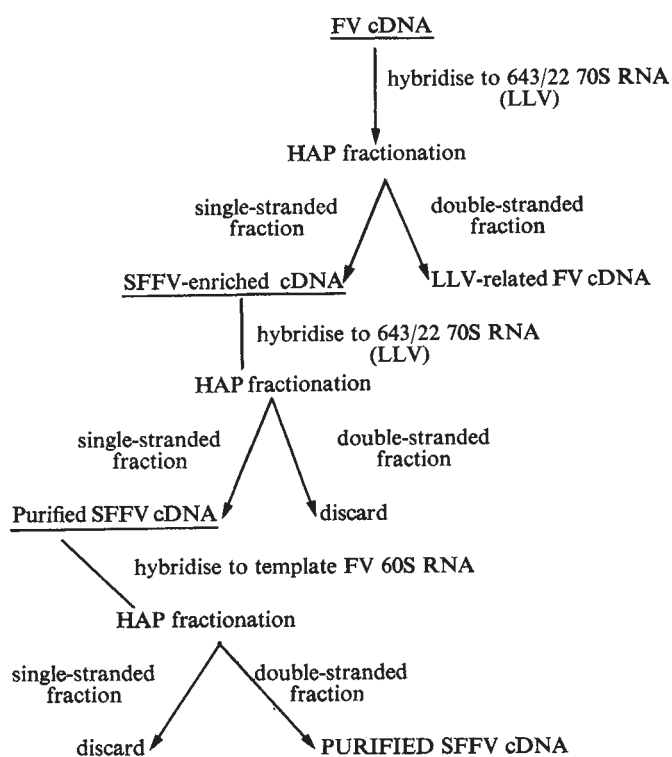


Fig. 2 Purification scheme: Isolation of SFFV cDNA from FV cDNA NB tropic-virus was prepared from uninduced F4-6 cells as described previously (refs 5, 14 and I.B.P., W.O., J.P. and R. Williamson, unpublished results). FV cDNA was prepared using lysed virions¹⁶ with modifications required for efficient transcription¹⁷. LLV-F 70S RNA was extracted from virus isolated from clone 643/22 cells and F4-6 60S RNA was isolated from F4-6 virus as described previously (refs 5, 14 and I. B. P., W.O., J.P. and R. Williamson, unpublished). Our standard hybridisation conditions were used^{5,18,19} and in both cycles of purification LLV-F 70S RNA was in a 25:1 excess over the FV cDNA and incubated such that hybridisation was complete (that is to $\text{Rot} = 10$)^{5,18}. The hydroxyapatite fractionation procedure was based on that previously published for 'sarc' cDNA purification²⁰. The purified SFFV cDNA was desalted by chromatography on Sephadex G50 and stored in water at -20°C .

tion to 60S RNA of the FV complex (Fig. 2). The purified SFFV-specific cDNA anneals more than 80% to the 60S RNA of the FV complex and less than 10% to the helper virus 70S RNA (Table 1).

The relatedness of the total FV cDNA and the purified SFFV cDNA to various murine leukaemia virus isolates was tested (Table 1). The unfractionated FV cDNA does contain 25% xenotropic- and 33% AKR ecotropic-related sequences. We have also tested the relatedness of the FV cDNA probe to the AKR and xenotropic virus genomes by measuring the protection of the 32 P-70S RNA by excess FV cDNA hybridisations. Only 10–15% of the xenotropic and 20% of the AKR virus genome was stabilised by hybridisation to excess FV cDNA (data not shown). Thus, the complete genome of neither the endogenous ecotropic AKR virus nor the xenotropic MLV is released by non-differentiating Friend cells. All of the sequences related to xenotropic or ecotropic genomic RNA thus seem to be homologous to the helper LLV-related sequences and not to the SFFV cDNA (Table 1). We do not rule out the possibility, however, that xenotropic sequences are present in the SFFV genome, but in the common region homologous to LLV-F and would therefore not be expected to be present in our highly purified SFFV cDNA. The spleen focus-forming virus-specific part of the genome of Friend virus of non-induced transformed

Table 1 Homology of various FV cDNA probes with murine leukaemia viral genomes and cellular RNA of normal erythroid cells

RNA	% cDNA stabilised by excess RNA (at saturation)		
	FV cDNA	LLV-related cDNA	SFFV cDNA
FV 60S	90	81	83
LLV 70S	70	73	5
AKR 70S	33	34	0
Balb -X	25	32	9
C3H-X	NT*	NT	5
Foetal nRNA	36†	17†	22†
Foetal liver nRNA	40†	29†	19†

Virus was isolated from F4-6 cells replicating Friend virus, from NIH 3T3 cells replicating AKR ecotropic virus and two CCL64 mink lung fibroblast lines replicating BALB/c and C3H xenotropic viruses. 60-70S RNA was isolated by standard procedures^{14,21}. The various cDNA preparations (prepared as described in Figs 1 and 2) were titrated with 60-70S RNA extracted from various virus isolates. The plateau values obtained in these titrations are presented, being the maximum amount of input cDNA hybridised by RNA excess. Nuclear RNA from 14-d mouse fetuses (minus livers) and 14-d mouse foetal livers was extracted by protease K treatment of citric acid nuclei²² and followed by CsCl centrifugation²³. Hybridisations were carried out such that all reactions were complete^{5,18,19} and hybrid formation was detected by S-1 nuclease analysis.

*NT, not tested.

†We calculate from these titrations in a number of experiments that the amount of FV cDNA related sequences is approximately 0.0006%–0.005%. Saturation values were obtained in the range of RNA–cDNA ratios of 80,000–100,000:1.

‡The values represent the hybridisation obtained at an RNA–cDNA ratio of 80,000:1, shortage of material did not enable us to obtain the full saturation obtained with the total FV cDNA.

erythroid cells thus seems to lack RNA sequences which are related to xenotropic MLV. This is in contrast to the high proportion of xenotropic RNA-related sequences in SFFV specific sequences of FV released by fibroblast cells⁸. Our data agree, however, with those of Ikawa *et al.*¹⁵ who used a different approach to isolate SFFV-cDNA.

In order to test a hypothesis that the basis for SFFV specificity may lie in viral transcription of sequences homologous to those involved in the normal process of differentiation, we hybridised nuclear RNA from normal erythroid cells with the purified SFFV cDNA (Table 1). These experiments detect sequences homologous to the unfractionated FV cDNA and SFFV cDNA both in RNA extracted from 14-d mouse foetal livers and in total foetal nuclear RNA (Table 1). Therefore we can conclude that there are no significant differences in the expression of sequences related to the SFFV probe in the erythroid and non-erythroid tissue (Table 1). The significance of these low levels of transcription of virus-specific information remains to be elucidated.

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Differences between the intracellular polypeptides of measles and subacute sclerosing panencephalitis virus

MEASLES virus is thought to be responsible for the rare childhood disease subacute sclerosing panencephalitis (SSPE)^{1–4}, which is a slowly progressive degenerative disease of the central nervous system occurring several years after an acute episode of measles. The physical and serological characteristics of SSPE virions isolated following cocultivation of infected brain cells bears a striking similarity to conventional measles virus^{1,2}. Some SSPE strains, however, have been found to differ from measles virus in biological properties and RNA homologies^{5–10}. There is also a report comparing the polypeptides of measles and SSPE virions in which an aberrant migration of the M polypeptide was noted¹¹. Unfortunately, no polypeptide profiles were presented and better documentation of the finding has not been published. Several theories have been advanced to explain the mechanism by which measles virus can persist and produce SSPE. These include an abnormal host immune response to a measles virus infection, a second viral agent acting in co-infection with a measles-like virus, and a mutation of the parental measles virus^{12,13}. In order to gain further insight into the nature of SSPE, we have compared the intracellular polypeptides of five SSPE isolates with those of measles virus.

CV-1 cells infected with the wild type (wt) Edmonston strain of measles virus contain six major viral-specific polypeptides²⁸. These have been termed G (surface glycoprotein), NS1 and NS2 (two nonstructural polypeptides), P2 (a phosphoprotein presumably associated with the nucleocapsid), NP (the major nucleocapsid protein), and M (the glycosylated membrane protein). These polypeptides, with the exception of NS1 and NS2, correspond to polypeptides that are seen in purified virions^{14–16}. Three additional virion polypeptides, L, P4 and P5 cannot usually be seen in extracts of infected cells. Furthermore, when cells are fractionated into nuclear and cytoplasmic fractions, the G, NS1 and NS2 polypeptides are absent from the nuclear fraction (ref. 28 and Fig. 1).

To compare the SSPE virus with measles, CV-1 cells were infected with either the Edmonston strain of measles or with one of five different SSPE strains (Hallé, McClellan, Mun-HT, Fisher or Mantooth). At 20 h post-infection, the cells were labelled with ³⁵S-methionine for 2 h, and nuclear and cytoplasmic fractions were prepared (Fig. 1). The major polypeptides previously noted with measles virus are seen in cells infected with all of the SSPE strains except Mun-HT. Although the general patterns of SSPE and measles polypeptides are similar, a close examination of Fig. 1 reveals a slight difference in at least one viral polypeptide, P2. In four of the SSPE strains, this nucleocapsid-associated phosphoprotein has a more rapid electrophoretic migration than that of the P2 polypeptide of the wt measles strain. Identical migrational differences can be seen in both the nuclear and cytoplasmic fractions. Although it is not clearly seen in Fig. 1, there is also the suggestion that differences in migration

exist among the M polypeptides. To determine the reliability of these findings, electrophoresis was performed under various conditions that increase the resolution of the P2 and M regions, and on samples which were labelled at later times to determine whether polypeptides synthesised by the Mun-HT strain (which produces cytopathic effects more slowly than wt measles and the other four SSPE strains) could be detected. When electrophoresis was carried out for periods longer than the 2 h described in Fig. 1, the variations in electrophoretic mobility of P2 and M were better resolved (Figs 2 and 3). In addition, when cells were infected with the Mun-HT strain and labelled 27 h post-infection (instead of 20 h), virus-specific polypeptides could be easily detected. The cytoplasmic fractions of cells infected with SSPE (Fig. 2) reveal: significantly faster migration of polypeptide P2 in all SSPE strains except Mun-HT, slightly slower migrations of the M polypeptides in the same four strains, and a much slower migration of the M polypeptide of the Mun-HT strain.

These differences in the rates of migration of the M polypeptide are even more dramatically illustrated in the nuclear extracts which were subjected to electrophoresis for an even longer period of time (Fig. 3). The striking abnormality of the M poly-

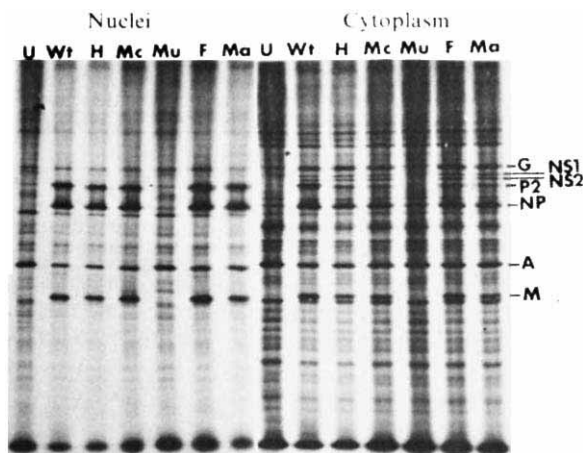


Fig. 1 Measles and SSPE virus specified intracellular polypeptides. Confluent monolayers of CV-1 cells in Falcon multicenter wells (Falcon 3008) (10^6 cells per well) were infected with virus at an input multiplicity of infection (MOI) of 50 plaque forming units per cell. After 2 h of adsorption at 37°C , the infected monolayers were fed with 1 ml of IMEMZO (International Biological Laboratories) supplemented with 10% foetal calf serum and then incubated at 37°C . At 20 h post-infection, cells were radiolabelled with ^{35}S -methionine in 1/2 ml methionine free Eagles MEM containing $100\ \mu\text{Ci } ^{35}\text{S}$ -methionine (specific activity $>400\ \mu\text{Ci Mm}^{-1}$). Before ^{35}S -methionine labelling, the monolayers were washed and preincubated for 1 h in methionine-free medium. Following 2 h of labelling, the medium was removed and the monolayers rinsed twice with ice cold TMN (0.01 M Tris, 0.0015 M MgCl_2 , 0.14 M NaCl, pH 7.2). The cells were then collected with a glass rod and suspended in 1 ml TMN. Cell suspensions were made 0.5% with respect to NP40 (NONIDET P40, Particle Data Laboratories) and incubated on ice for 30 min. The samples were then centrifuged at $1,000g$ for 1 min. The supernatant represents the cytoplasmic fraction and the pellet the nuclear fraction. The nuclei were suspended in 1 ml of TMN and 10 volumes of -20°C acetone were added to both fractions. Protein was precipitated overnight at -20°C and then pelleted at $1,500g$ for 2 h. The acetone was poured off and the pellets allowed to air dry for 24 h at room temperature. The pellets were then suspended in gel sample buffer containing 5% mercaptoethanol and 2% SDS. SDS-polyacrylamide gel electrophoresis was carried out according to the method of Laemmli²⁷. Electrophoresis was carried out in a 10% polyacrylamide slab gel at a constant current of 45 mA for 2 h. Gels were fixed in 50% methanol-7% acetic acid for 1 h, dried under vacuum and exposed for autoradiography using Kodak NS2T X-ray film. U, uninfected; Wt, Edmonston wt measles; SSPE strains: H, Hallé; Mc, McClellan; Mu, Mun-HT; F, Fisher; Ma, Mantooth. Nomenclature of viral polypeptides: G, glycoprotein; NS1, and NS2, nonstructural polypeptides 1 and 2, respectively; NP, nucleocapsid-associated phosphoprotein; M, nonglycosylated membrane protein; A, cellular actin.

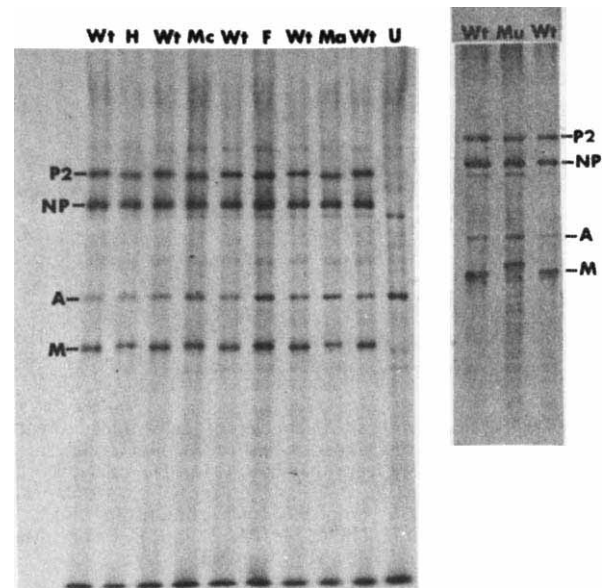


Fig. 2 Polypeptide synthesis in cytoplasmic fractions of cells infected with measles and SSPE virus. Experimental protocol is as described in the legend to Fig. 1, except that a second monolayer infected with the SSPE strain Mun-HT was labelled at 27 h instead of 20 h post-infection (far right). Electrophoresis was for 2½ h at 45 mA. U, uninfected; Wt, wt measles; SSPE strains: H, Hallé; Mc, McClellan; Mu, Mun-HT; F, Fisher; Ma, Mantooth.

peptide in the Mun-HT strain is easily seen, as are the smaller variations in the M polypeptides of the other strains. In these nuclear extracts, the four SSPE strains (Hallé, McClellan, Fisher and Mantooth) also exhibit the aberrantly migrating P2 polypeptides, as noted previously, while the P2 polypeptide from the fifth SSPE strain, Mun-HT, does not differ significantly from the P2 polypeptide of wt measles (Fig. 3).

These results show significant differences in the migration of the P2 and M polypeptides between the five SSPE isolates and wt Edmonston measles. The precise biochemical alterations causing these aberrant electrophoretic migrations are not known. In addition to the measles strain described in this report, we have recently obtained a second strain of Edmonston measles (R. Rustigian, personal communication). This strain has been maintained by passage through HeLa and Vero cells for more than 15 yr. An examination of the polypeptides of the latter strain has revealed migrations identical to those of the measles strain described here for all of the major polypeptides except P2 (Wechsler, unpublished results). Furthermore, Mountcastle and Choppin in a recent study of four different measles strains¹⁶ were also unable to detect migrational variants of the M polypeptide. Thus, while the P2 polypeptides of different measles strains may vary, no variants have been found in the M polypeptides from six different measles strains. Therefore, it is highly unlikely that the M polypeptide differences of the SSPE isolates described in this report reflect normal strain variations. Perhaps the most likely explanation for such altered polypeptide mobilities is mutation in the genome region coding for the M polypeptide. While single site, revertible mutations can cause electrophoretic migrational variations of individual polypeptides similar in magnitude to those exhibited by M^{17,18} it is not clear if this is the case, or if more complex explanations (multiple mutations or recombination) are in fact responsible for these changes.

Two additional lines of investigation support the potential significance of these findings: Hall and ter Meulen (ref. 19, and personal communication), have detected apparent alterations of the M polypeptide mRNA in SSPE isolates, and two cell lines persistently infected with measles virus^{20,21} have revealed aberrant M polypeptides (S.L.W., R.J. Rustigian and B.N.F., in preparation). These facts, and our finding that all five SSPE isolates contain aberrantly migrating M polypeptides, support



Fig. 3 Polypeptide synthesis in nuclei of cells infected with measles and SSPE virus. Experimental protocol is as described in the legend to Fig. 1, except that SSPE Mun-HT infected cells were labelled at 27 h instead of 20 h post-infection. Electrophoresis was for 3 h at 45 mA. U, uninfected; Wt, Edmonston wild type measles; SSPE strains: H, Hallé; Mc, McClellan; F, Fisher; Ma, Mantooh; Mu, Mun-HT.

the hypothesis that a mutation in the M protein gene is associated with, and could account for, the persistence of measles virus in the disease SSPE.

The current view that the nucleocapsid in SSPE does not align with the cell membrane²² and that the M polypeptide may mediate recognition between the viral nucleocapsid and the cell membrane during viral assembly²³⁻²⁶, lends further support to the hypothesis that an abnormal M polypeptide is responsible for SSPE and may help to explain the defective state of SSPE *in vivo*. In any event, the data presented here indicate that markers which can distinguish SSPE from measles virus now exist, and that the virus isolated from brains of patients with SSPE appears to differ from conventional measles virus by at least one and possibly two mutational changes.

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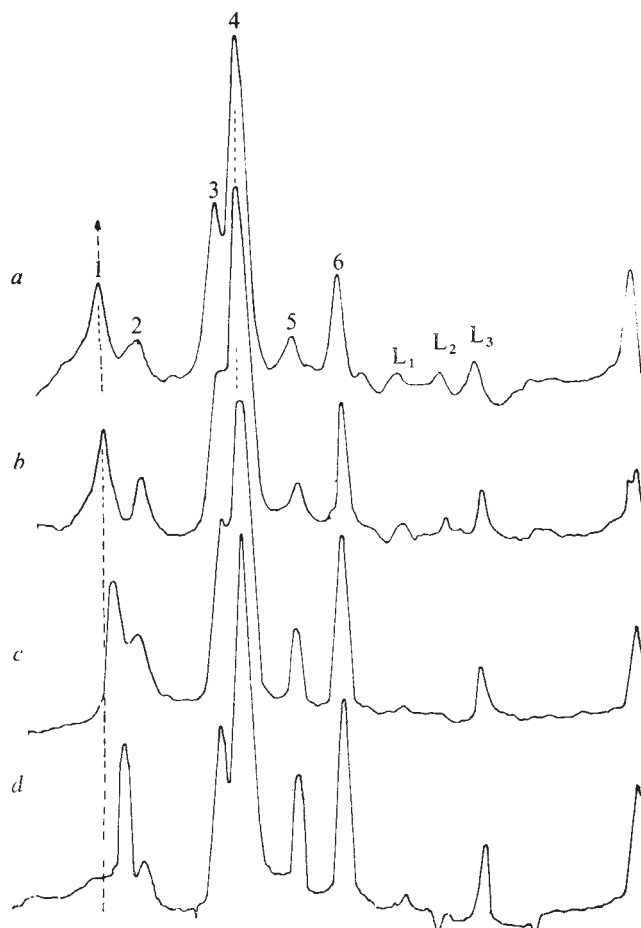
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Membrane proteins of subacute sclerosing panencephalitis and measles viruses

SUBACUTE sclerosing panencephalitis (SSPE) is a rare, slowly evolving disease of the central nervous system (CNS) which has been associated with measles virus infection. A measles-like virus (SSPE virus) has been isolated from SSPE brain and lymph node material. Also, patients with SSPE show a humoral hyperimmune reaction against measles virus. These findings implicate measles virus as a possible aetiological agent in this disease; however, they do not explain the pathogenicity of SSPE^{1,2}. Additional factors, either host or virus derived, must have a pathogenetic role, as rarity and rural prevalence of SSPE cannot be correlated to the ubiquitous measles virus infection². Indeed, biological, ultrastructural and biochemical investigations³⁻⁵ have indicated minor differences between SSPE and measles virus strains. On the basis of these findings, we have analysed the mRNAs of these viruses from infected cells and compared the antigenic properties of membrane (M) proteins isolated from purified SSPE (leukoencephalitis (LEC)) viruses with those of measles (Edmonston) viruses. The results presented here show differences among the M proteins as well as the pattern of mRNAs of these viruses. The findings allow differentiation between these viruses and indicate that SSPE viruses are related but not identical to measles virus.

Fig. 1 Electrophoresis of mRNAs on 2.5% polyacrylamide gels in denaturing conditions (97% formamide, 6 M urea) according to the method of Villareal *et al.*¹⁰ a, Attenuated Edmonston measles strain; b, Woodfolk, a wild measles strain; c, strain LEC SSPE; d, strain USC SSPE. Shown above are densitometer tracings of a fluorogram (migration of the mRNAs is from right to left). The RNAs were extracted from virus-infected Vero cells and purified as previously described⁵. Molecular weights were calculated relative to ribosomal RNA.



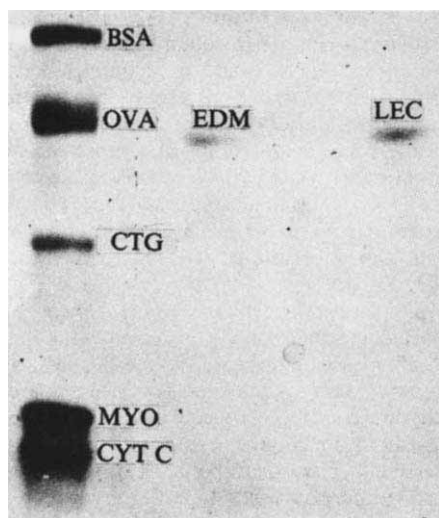


Fig. 2 Electrophoresis of purified M proteins of measles virus [strain Edmonston (EDM)] and SSPE virus (strain LEC) on 15% polyacrylamide gels. The conditions of electrophoresis were as described by Laemmli¹¹. For each M protein only one band can be observed, indicating a molecular weight of approximately 38,000. Protein markers were bovine serum albumin (BSA), ovalbumin (OVA), chymotrypsinogen (CTG), myoglobin (MYO) and cytochrome c (CYT C).

Recent investigations into the degree of genome homology between several measles and SSPE viral strains have shown that there are small differences in their genetic relatedness⁵. This observation is supported by studies of neutralisation kinetics which suggest a difference between several SSPE strains and wild-type measles virus³. In addition, a comparative study of the antigenicity of these viruses by applying a binding-inhibition test based on an indirect radioimmunoassay (IRIA) revealed similar antigenic differences^{6,13}. Evidence that some differences in structural protein(s) may exist between measles and SSPE viruses was first indicated in a report by Schluederberg *et al.*⁷ who showed that the M protein of one SSPE strain migrated more slowly than the M protein of measles virus. These findings have been confirmed and extended by Wechsler and Fields⁴ who have shown that five strains of SSPE virus all contain M proteins of higher molecular weights than does the wild-type measles virus. In order to locate these differences, we have analysed the mRNAs of these viruses by gel electrophoresis, the idea being that since a difference in the overall genetic content exists, it should be evident in at least one of the complementary mRNAs.

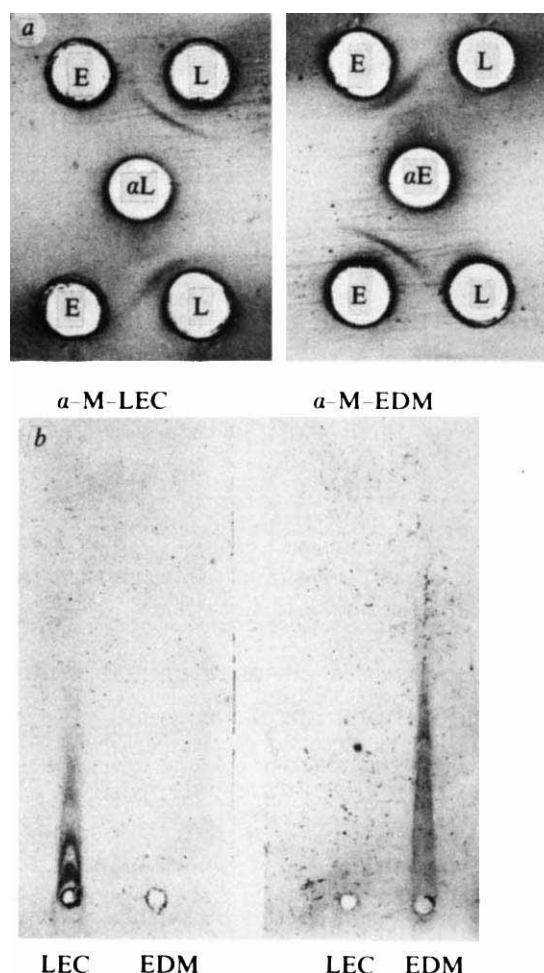
Figure 1 shows densitometer tracings of fluorographs of the mRNAs of two measles and two SSPE viruses. A total of six major mRNA species could be resolved and the minor, more slowly migrating bands with large molecular weight RNAs (L1 L2 L3) are assumed to represent aggregation products or an additional large mRNA. It can be seen that all the RNAs with the exception of RNA 1 had identical mobilities. In the case of the SSPE mRNA, the RNA 1 migrated more slowly, indicating a higher molecular weight. Since this RNA is considered to be the mRNA for the M protein of virus, it was assumed that the differences between the mRNA 1 of these viruses could reflect differences of the M proteins.

To investigate this aspect further, M protein was isolated from purified SSPE (LEC) and measles (Edmonston) viruses which had been grown in Vero cells using a minor modification of the method for paramyxoviruses described by Scheid *et al.*⁹. Figure 2 shows the electrophoretic pattern of the purified proteins on 15% polyacrylamide gels. For each M protein only one band was observed having a molecular weight of around 38,000.

In order to compare the antigenicity of the purified M proteins, antisera were produced in rabbits. In standard serological assays for measles virus such as haemagglutination inhibition, CF or NT tests, no antibody activity against Edmonston or LEC virus could be detected. However, using the isolated M proteins of both viruses as antigen in an IRIA, high antibody titres up to 2^{-15} were measurable only with the corresponding antiserum. Immunodiffusion and immunoelectrophoresis were used to compare further the antigenic relatedness or differences between the two M proteins. As demonstrated in Fig. 3a, only in the homologous system was a precipitation line observed. Antigenic differences were further documented by immunoelectrophoresis (Fig. 3b). An antigen-antibody reaction resulting in a 'rocket' occurred exclusively in the homologous system whereas the heterologous antigen did not form an immunoprecipitation.

The results reported here provide the basis for a differentiation of SSPE and measles virus. Comparison of the mRNAs synthesised in infected cells indicates that the

Fig. 3 a, Immunodiffusion according to the Ouchterlony technique. Precipitation lines are only detectable in the homologous antigen-antibody system. E, Edmonston M protein; L, LEC M protein; α E, anti-Edmonston M protein; α L, anti-LEC M protein. Both antisera are prepared in rabbits which were injected twice with purified M protein. The first injection consisted of M protein mixed with Freund's complete adjuvant. Booster injections, 6 weeks later, were carried out with M protein alone. **b**, Rocket immunoelectrophoresis according to the method of Laurell¹². A precipitation occurs only in the homologous antigen-antibody system. LEC, LEC M protein; EDM, Edmonston M protein; α M-LEC, rabbit antiserum against LEC M protein; α M-EDM, rabbit antiserum against EDM M protein. Each antigen well obtained 1,000 antigen units as determined by a solid phase indirect radioimmuno assay¹³.



mRNA for the SSPE M protein is larger than that of the measles mRNA. This may well account for the previously reported differences in genome homology, as well as for the seemingly larger molecular weight of SSPE M protein. Moreover, the M proteins of the two viruses studied are antigenically different, demonstrating that SSPE virus is, in fact, not identical with measles virus. These findings raise an interesting point concerning the aetiology of SSPE. Epidemiological studies have shown that SSPE patients do have acute measles at an early stage in life, which clinically does not seem to be different from regular measles². The chronic CNS disease developing on average between 7 and 8 yr after acute measles suggests a persistency of measles virus in the CNS during the incubation period.

If SSPE viruses are derived from measles virus, one may speculate that some genetic changes, in particular one affecting the gene for M proteins, leads to an altered biological property, that is, a persistent infection. Alternatively, it is conceivable that SSPE viruses represent independent strains of measles virus group infecting SSPE patients in the first place. However, epidemiological data would not support the latter interpretation since a clustering of this disease has not been observed.

In conclusion, it must be stated that one limitation in this study was the use of only two viruses in the antigenic comparison. There is a possibility that the results might be a chance observation reflecting differences between the rabbits immunised or successive mutations of the SSPE LEC virus, which are not typical for the other SSPE strains. Because of the difficulties in obtaining large amounts of purified virus material and purified M proteins from other SSPE and measles strains, the present antigenic analysis did only include SSPE LEC and the Edmonston strain of measles virus. Further studies will include other measles and SSPE strains.

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Rapid stimulation of protein carboxymethylation in leukocytes by a chemotactic peptide

THERE has been considerable interest recently in protein carboxymethyltransferase, and in its possible role in secretory¹ and chemotactic mechanisms^{2,3}. Using S-adenosyl-L-methionine as the methyl donor, this enzyme catalyses the formation of protein methyl esters^{4,5} which undergo rapid spontaneous hydrolysis at physiological pH to liberate methanol^{6,7}. The enzyme is widely distributed in mammalian tissues, especially

in endocrine organs and brain^{7,8}. *Escherichia coli* and *Salmonella typhimurium* appear to require methylation for chemotaxis^{2,3}. Eukaryotic cells, such as leukocytes (neutrophils, polymorphonuclear cells), have been shown to respond chemotactically to a variety of peptides derived from complement⁹, to crude bacterial factors¹⁰, and more recently to well-defined synthetic formylated peptides¹¹. We wish to report here the presence in leukocytes of a protein carboxymethyltransferase system, whose activity is specifically stimulated by synthetic peptides, which may be related to chemoattractants produced by bacteria¹².

Neutrophils were taken from rabbit peritoneal exudates, 8 to 12 h after intraperitoneal injection of 0.1% glycogen in normal saline¹¹. Chemotactic responsiveness of each cell preparation was assayed as described elsewhere⁹, using a modified Boyden chamber procedure. Protein carboxymethylase was measured in rabbit neutrophils by procedures previously described¹. To assess the correlation between chemotaxis and protein carboxymethylation, the cells obtained from peritoneal exudates were separated by centrifugation at 400g for 10 min and gently resuspended in Gey's balanced salt solution, pH 7.2, containing 0.015 M Hepes and 2 units per ml of heparin. After preincubation for 5 min at 37 °C, protein carboxymethylation was measured as described in Fig. 1. In some experiments, ³H-methanol liberated during incubation from protein methyl esters was isolated and measured as follows: 1 ml of 70% Dowex 50 W-X-8 resin

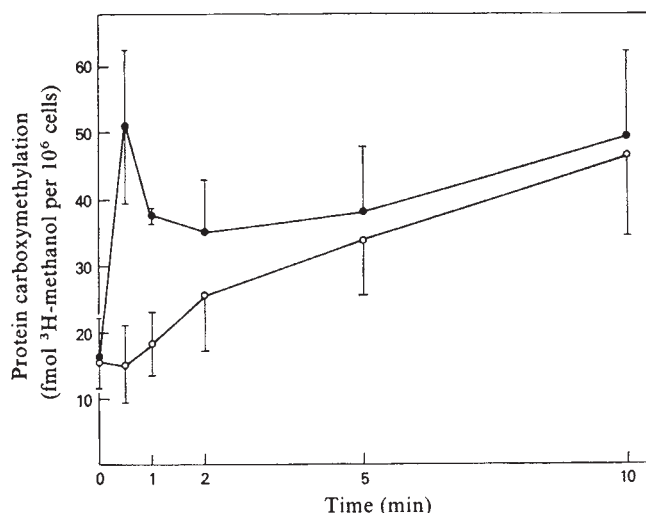


Fig. 1 Stimulation of polymorphonuclear (PMN) cell protein carboxymethylation by fMet-Leu-Phe. After preincubation at 37 °C for 5 min, the reactions (5 to 10 × 10⁶ cells per ml) were initiated with the simultaneous addition of 10 μCi of ³H-L-methionine (final concentration 6.7 × 10⁻⁷ M) and peptide (10⁻⁸ M) in Gey's solution (●), in a final volume of 1.0 ml. Control tubes were also incubated in the absence of peptide (○). All reactions were terminated by the addition of 1.0 ml of cold 10% trichloroacetic acid and protein carboxymethylation measured by a modification of procedures previously described¹. After centrifugation for 10 min at 20,000g, the precipitated esters formed from endogenous proteins were hydrolysed with 300 μl of 1.0 M borate buffer at pH 11.0, containing unlabelled methanol (2.6% v/v) as carrier. The hydrolytic product, ³H-methanol, was extracted with 3 ml of a 3:2 mixture (v/v) of toluene and isoamyl alcohol. After centrifugation for 10 min at 9,500g, two 1-ml portions of the organic phase were transferred to vials. The radioactivity in one was determined after the addition of Aquasol (New England Nuclear Corp.). The ³H-methanol in the second vial was evaporated for 1-2 h at 80 °C, and the residual, nonvolatile radioactivity measured. The difference in radioactivity in samples before and after evaporation was taken as a measure of carboxymethylation. The above data represents the mean ± s.e.m. for triplicate determinations in three separate experiments.

*Significantly greater than control, with $P < 0.05$, by Student's t test (two-tailed).

in 10% trichloroacetic acid was added to 1 ml of the protein-free supernatant obtained after centrifugation of the acid-extracted reaction mixture. After 15 min agitation the resin was separated by centrifugation at 1,100g for 15 min and 10 M NaOH was added to bring the pH to 11. One ml of the supernatant was extracted with 6 ml of (3:2) toluene: isoamyl alcohol, and ^3H -methanol determined as described in Fig. 1. Control assays in all experiments consisted of incubations in the absence of the peptide.

The potent chemotactic peptide fMet-Leu-Phe was prepared by Dr F. Freer *et al.* under Contract No. NO1-DE5-2477 with NIH. The dipeptide Phe-Met was obtained from Research Plus Labs, Denville, N.J., and formylated as described by Sheehan and Yang¹³. t-Butyloxycarbonyl-L-Phe-D-Leu-L-Phe-D-Leu-L-Phe (t-Boc-PLPLP) was a gift from Dr E. Gross, NICHD, NIH. All other chemicals were obtained from commercial sources.

Protein carboxymethylase was found to be present in neutrophils and had a specific activity similar to the platelet enzyme and greater than that measured in red blood cells¹⁴.

The simultaneous addition of the leukoattractant fMet-Leu-Phe (10^{-8} M) and ^3H -methionine to intact cells resulted in a rapid two- to threefold increase in protein methylester formation (Fig. 1), which seemed to be maximal at 30 s, the shortest incubation time measured. The accelerated methylation stimulated by the peptide lasted only 5 min (Fig. 1). In the absence of the peptide the cells showed a linear increase of protein carboxymethylation with time.

Preliminary evidence indicates that the chemoattractant selectively stimulates the protein carboxymethylation system. The uptake of ^3H -L-methionine into neutrophils, itself a rapid process, was not increased by fMet-Leu-Phe (R. F. O'Dea, in preparation). During the first minute after addition of chemoattractant, the mean increase in protein carboxymethylation as compared to control was four times greater than the changes in total ^3H incorporation into protein (Table 1). Evidence that this protein carboxymethylation does not depend upon *de novo* protein synthesis was obtained by the use of cycloheximide. After addition of the chemotactic peptide, the usual two- to threefold increase in carboxymethylation was found, whereas ^3H -incorporation into protein was nearly abolished (Table 1). At these levels of cycloheximide, chemotaxis is not affected, indicating that the directed migration of these cells is independent of protein synthesis.

Table 1 Stimulation of neutrophil protein carboxymethylation but not protein synthesis by a leukotactic peptide

Effect	% of control	
	cycloheximide absent	cycloheximide added
Protein carboxymethylation	217 ± 23 (8)	232
^3H -incorporation into protein	124 ± 7 (8)	22

Incubation and assay conditions for protein carboxymethylation were identical to those described in the legend to Fig. 1, except that protein carboxymethylation reactions were terminated 60 s after addition of ^3H -L-methionine and fMet-Leu-Phe (10^{-8} M). Cycloheximide ($100 \mu\text{g ml}^{-1}$) was present throughout the incubation period where indicated. To assess the incorporation of ^3H into protein, 100 μl of the trichloroacetic acid-treated reaction mixture was diluted 10-fold with 10% trichloroacetic acid containing 10^{-3} M unlabelled L-methionine, heated to 90 °C for 15 min to solubilise nucleic acids and filtered over Whatman GF-B glass fibre filters, previously washed with 10% trichloroacetic acid. The filters were subsequently washed three times each with 1.0 ml 10% trichloroacetic acid, three times with 2.0 ml ethanol and three times with ethanol:diethyl ether (1:3). The residual bound radioactivity was counted in 10 ml of Aquasol. All values represent the mean % change $\pm$ s.e.m., relative to control, for the number of separate experiments indicated in parentheses. The values obtained in the presence of cycloheximide presents the average % change for five separate determinations in a representative experiment. Control value (minus fMet-Leu-Phe) for ^3H -methionine incorporation into protein was approximately 20 pmol per 10^6 cells and for protein carboxymethylation approximately 20 fmol per 10^6 cells of ^3H -methanol liberated.

Table 2 Inhibition of stimulated protein carboxymethylation by non-chemotactic antagonists

	f-Phe-Met (10^{-4} M)	% of control t-Boc-PLPLP (5×10^{-6} M)
Control	100	100
10^{-8} M fMet-Leu-Phe alone	142 ± 0.9*	130 ± 8.2*
Antagonist alone	92 ± 7.9	98 ± 5.2
fMet-Leu-Phe plus antagonist	89 ± 4.2	92 ± 2.2

Incubation and assay conditions for protein carboxymethylation were identical to those described in the legend to Fig. 1, except that all reactions were terminated 60 s after addition of ^3H -L-methionine and fMet-Leu-Phe. Antagonist peptides were present during both the preincubation and incubation phases. Each value shown represents the mean $\pm$ s.e.m. for the % of control response for triplicate values in two different experiments. Control activities for protein carboxymethylation were 23 and 68 fmol of ^3H -methanol liberated per 10^6 cells for the experiments performed with fPhe-Met and t-Boc-PLPLP, respectively.

*Significantly greater than all other groups, with $P < 0.05$, by Student's *t* test.

We have also compared the increase in protein carboxymethylation stimulated by fMet-Leu-Phe to alterations in the incorporation of ^3H -methionine into the nonvolatile fraction which is extracted into toluene:isoamyl alcohol (3:2). In a large series of experiments, fMet-Leu-Phe (10^{-8} M) produced a twofold greater increase in protein carboxymethylation within one minute, as compared to methylation into a non-volatile fraction. These changes in incorporation in a non-volatile fraction of cells after stimulation by fMet-Leu-Phe were inconsistent and poorly correlated with changes in carboxymethylation.

Additional evidence in support of the specificity of the peptide-stimulated increase in carboxymethylation was obtained in experiments with the chemotactic antagonist peptides, fPhe-Met and t-Boc-PLPLP (Table 2). These compounds have previously been shown to inhibit specifically the chemotactic response of neutrophils to peptide agonists^{15,17}. Both fPhe-Met (10^{-4} M) and t-Boc-PLPLP (5×10^{-6} M) completely blocked the specific protein carboxymethylation promoted by fMet-Leu-Phe (10^{-8} M). Neither antagonist alone had any effect on the basal level of protein carboxymethylation. Thus, this response probably reflects an increased carboxymethylation of cellular proteins specifically initiated by interaction of neutrophils with the chemotactic peptide. The transient, and presumably labile nature of this methylation is supported by the observation of a concomitant increase in ^3H -methanol liberated from the methylated protein which accompanied the peak formation of protein ^3H -methyl esters (R. F. O'Dea *et al.*, in preparation). In about 15% of the batches of cells, it was not possible to demonstrate a peptide-stimulated carboxymethylation. These cells were also chemotactically inactive. These observations provide additional evidence that there is a high correlation between the cell's chemotactic responsiveness and its ability to carry out a specific carboxymethylation.

Aswanikumar *et al.*¹⁸ reported that fMet-Leu-Phe specifically inhibits the binding of a radiolabelled peptide agonist to neutrophil membranes with an ID_{50} of 3×10^{-10} M. This value is similar to the ED_{50} reported by Showell *et al.*¹¹ for fMet-Leu-Phe as an agonist in neutrophil chemotaxis. Maximal protein carboxymethylation of neutrophils occurred at 10^{-8} M fMet-Leu-Phe, with an approximate ED_{50} of 1×10^{-9} M (Fig. 2). Although this value is somewhat higher than the above calculated values, the shape of the dose-response curve is similar to the chemotactic dose-response curves reported for a number of other peptides¹². At high concentrations of the peptide there is an inhibition of both chemotaxis¹² and protein carboxymethylation (Fig. 2). It is also possible that both the absence of a defined gradient and the larger numbers of cells contributed to an observed higher ED_{50} for a protein carboxymethylation stimulation by fMet-Leu-Phe.

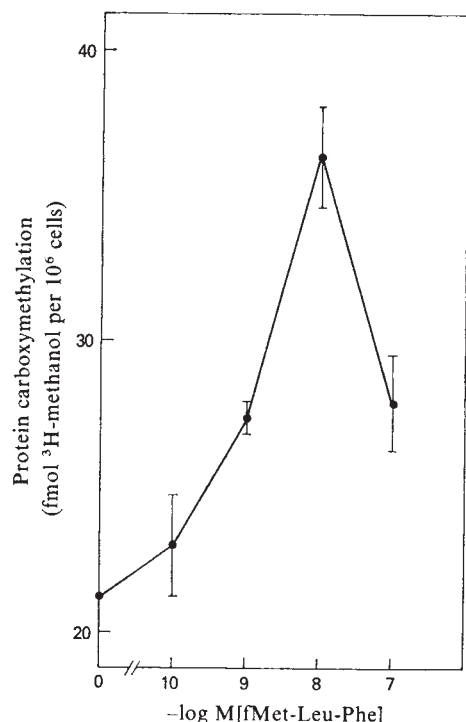


Fig. 2 Effect of varying concentrations of f-Met-Leu-Phe on polymorphonuclear cells (PMN) protein carboxymethylation. The conditions employed in these experiments were identical to those described in the legend to Fig. 1, except that all reactions were terminated 60 s after the addition of peptide and ³H-L-methionine. Each point represents the mean $\pm$ s.e.m. of triplicate determinations in two separate experiments.

Previous studies with synthetic formylated chemotactic peptides have indicated a role for peptidases¹⁵ in leukocyte chemotaxis, and have contributed to the more recent demonstration of a specific chemotactic receptor on the neutrophil¹⁶. The interaction of attractant with receptor presumably generates a signal which ultimately results in directed motion of the cell. Such motile behaviour in phagocytic leukocytes is of critical importance in the defence of the host against invading organisms. In bacteria, protein methylation has been shown to participate in their directed motility to certain chemical stimuli^{2,3}. It seemed reasonable, then, that an efficient biochemical process for such types of behaviour might in part be conserved in evolution. The demonstration of an enhanced rate of neutrophil protein carboxymethylation after exposure to chemoattractant is consistent with this suggestion, and indicates that the transduction of the chemotactic stimulus into motile behaviour involves protein carboxymethylation. Enzymatic esterification by methylation neutralises the negative charge of the carboxyl groups of protein. Such neutralisation of charge can alter the conformation of membrane proteins and thus initiate events leading to chemotaxis of leukocytes. The response described here is sensitive, specific, rapid, transient and not dependent on *de novo* protein synthesis, characteristics often associated with transduction of a biological signal.

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Human foetal palatal corticoid receptors and teratogens for cleft palate

CLEFT secondary palate can be induced experimentally in various mammalian species by the administration of glucocorticoids to pregnant animals¹⁻⁴. The well known differences in susceptibility to cortisone-induced cleft palate in inbred strains of mice have been explained in terms of genetic differences in foetal facial mesenchymal glucocorticoid receptor levels^{5,6}. The quantity of one protein in particular, with an isoelectric point (*pI*) of approximately 7.0, seems to be correlated rather closely with the susceptibility of various strains to cortisone-induced cleft palate⁶. It has also been shown that much of the difference in cleft palate susceptibility between C57BL/10 (B10) and A/J mice can be accounted for by *H-2*-linked genes^{7,8}, and that there is *H-2*-linked variation in the level of cortisol-binding proteins obtained from the cytosol fraction of foetal palatal tissue⁶. As corticoids form a specific cytoplasmic cortisol receptor complex in target tissues which interacts with the genome resulting in activation or depression of transcription^{9,10}, a product of a gene in or near the *H-2* locus seems to be the glucocorticoid receptor, the level of which has to be raised for cleft palate to occur. As a first step to determine whether such a mechanism may operate in humans, we have examined whether human palatal mesenchymal cells, obtained in cultures from foetuses shortly after the critical period of secondary palatal closure, contain glucocorticoid receptors. We show here that these cells have specific glucocorticoid receptor proteins with high affinity for dexamethasone and triamcinolone acetonide, and that dexamethasone is displaced from its binding sites only by drugs which produce cleft palate in sensitive mouse strains. Thus, the human foetus contains an active glucocorticoid receptor mechanism immediately after the critical period of palatal organogenesis, which may be involved in palatal clefting.

Human foetuses weighing 90 and 150 g (at approximately 13 and 15 weeks of gestation) were obtained under aseptic conditions from therapeutic abortions. Secondary palates were minced with scalpels and pieces of approximately 1 mm² were seeded into 25 mm² plastic flasks containing Eagle's basal medium, 1 $\times$, (BME) with Hank's salts (Flow Labs), 10% foetal bovine serum (Sterile Systems, Inc.), penicillin, 0.6 units ml⁻¹, streptomycin, 0.6 μ g ml⁻¹, (Gibco), essential amino acids 1 $\times$, and 4 mM glutamine (Microbiological Associates). After several passages in plastic flasks, the cells were transferred and grown in disposable glass roller bottles, 690 cm growth area, (Bellco) at 37 $^{\circ}$ C. Cultures of mesenchyme cells obtained from secondary palates seemed to have fibroblastic morphology. Cells for receptor studies were used after a total of 7-10 passages. In the present study, we used the synthetic glucocorticoid, dexamethasone, rather than cortisol, because dexamethasone is more tightly bound to the receptor¹¹, binds poorly to ligandin¹¹, does not bind to transcortin¹², and stabilises the receptor¹³.

In all these experiments, there were no essential differences in the various experimental procedures between the findings with the 90-g and the 150-g fetuses, and so the results presented are those from the 90-g fetus. Figure 1 indicates specific binding of ^3H -dexamethasone or ^3H -triamcinolone acetonide to whole-cell suspensions of the human foetal mesenchymal cells grown *in vitro* measured as a function of the free steroid concentration. The curves indicate the presence of specific corticosteroid receptors for dexamethasone and triamcinolone acetonide in these cells, which approach saturation between 9×10^{-9} M and 10×10^{-9} M of either steroid. Scatchard plots of the data (Fig. 1) are linear for both steroids, indicating the presence of a single class of binding sites with an apparent K_d for dexamethasone of 3.3×10^{-8} M and a K_d for triamcinolone acetonide of 4.1×10^{-8} M.

Virtually all of the radioactivity bound tightly to protein in the nuclei of the cultured cells after 40 min incubation was unmetabolised ^3H -dexamethasone (Fig. 2). A typical micro-electrofocussed pattern of ^3H -dexamethasone-binding proteins in the cultured foetal palatal cell cytosol is shown in Fig. 3. The pattern shows a peak of ^3H -dexamethasone-binding protein at pI 7.2 which is absorbed by DNA. Since most investigators agree that the glucocorticoid receptors are absorbed by either chromatin from the nuclei of the same tissue, or by calf thymus DNA (refs 12, 13), this pI 7.2 ^3H -dexamethasone-binding protein satisfies this criterion for a glucocorticoid receptor.

Table 1 shows that the binding of ^3H -dexamethasone to human foetal palatal cells is depressed specifically only by other

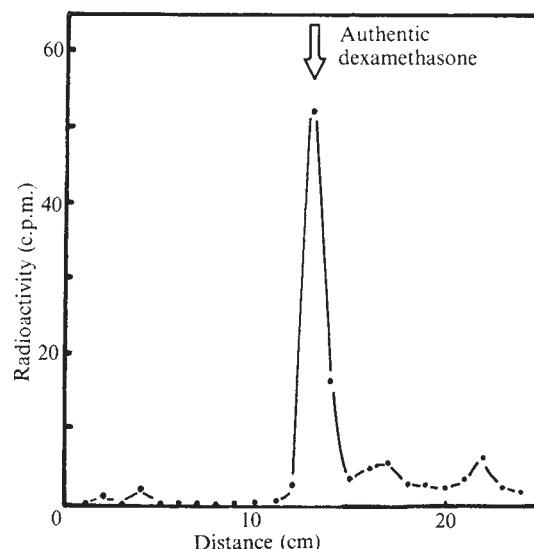


Fig. 2 Nuclear incorporation of ^3H -dexamethasone by cultures of human foetal palatal cells. Cultured cells were incubated *in situ*, in roller bottles, for 40 min with 10 nM of a ^3H -dexamethasone-containing culture medium without foetal bovine serum. The cultures were then washed of excess ^3H -dexamethasone and harvested with trypsin (0.25%). Cell nuclei were purified by the method of Maggio *et al.*²⁷. Radioactive material incorporated into the nuclei was extracted with methylene dichloride and was chromatographed on the TLC sheet with cold authentic dexamethasone. Developer (Benzene-formamide, 9:1)

corticoids and testosterone. The Table also shows that the binding of ^3H -dexamethasone is rather severely depressed by phenytoin, chlorpromazine, and sodium phenobarbital, drugs which produce cleft palate in the corticoid-sensitive A/J mouse strain^{14,15}; binding was reduced to a much lower degree by drugs which have little cleft palate teratogenicity in the A/J strain, namely, diazepam and sodium barbital¹⁴, and not at all by drugs which do not produce cleft palate in sensitive strains,

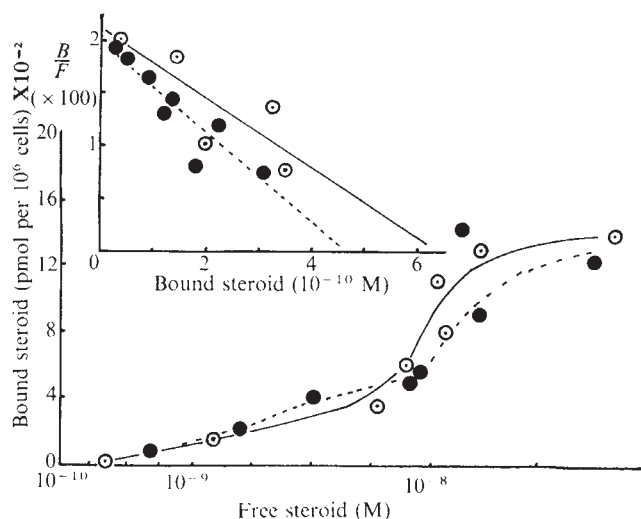


Fig. 1 When the cells were confluent in the roller bottles, they were washed with 20 ml BME without foetal bovine serum and dissociated with trypsin (0.25%). Glucocorticoid-receptor activity was measured in whole-cell suspensions (10^6 cells ml^{-1} counted in a haemocytometer using Trypan blue) using ^3H -dexamethasone, 37.6 Ci mmol^{-1} , or ^3H -triamcinolone acetonide, 33.7 Ci mmol^{-1} (New England Nuclear), according to the method of Baxter *et al.*²⁵, as modified by Sibley and Tomkins²⁶. Aliquots of 0.5 ml were incubated at 37 °C for 1 h in a mixture containing 1–60 nM ^3H -steroid in the absence or presence of an excess of unlabelled steroid (10^{-8} M) in quadruplicate assays to correct for nonspecific binding²⁵. Cell suspensions were incubated with shaking at 37 °C. Following incubation, tubes were chilled to 4 °C and cells were pelleted at 800g for 1 min. The pellets were washed twice with 1 ml physiologically balanced Tris buffer and finally resuspended in 1 ml distilled H_2O . Samples were then sonicated at a setting of 35 W for 15 s each, using a heat system (Ultrasonics Inc. Sonifier Cell Disruptor Model W185D). Aliquots were then dissolved in 5 ml of scintillation fluid Formula 950 (New England Nuclear) and counted in a Packard TriCarb 3375 liquid scintillation spectrometer at 25% efficiency for ^3H . Specific binding is the total binding minus the binding which is not displaced by excess ($10 \mu\text{M}$) steroids. Specific binding is saturated by about 10^{-8} M. $\circ$, ^3H -dexamethasone; $\bullet$, ^3H -triamcinolone acetonide. Inset shows the same data transformed to Scatchard plots. (B , specific binding in pmol per 10^6 cells; F , ^3H -steroid concentration in pM).

Table 1 Effect of unlabelled drugs on binding of ^3H -dexamethasone to human foetal palatal cells

Drugs	Binding (% control)	Produces (+) cleft palate in A/J mice	Ref.
Cortisol	20.3		
Corticosterone	47.0		
Cortexolone	76.0		
Testosterone	20.5		
17 β -Oestradiol	92.1		
Pregnenolone	97.8		
Progesterone	100.0		
17-Hydroxyprogesterone	100.00		
Phenytoin	24.8	+	15,23
Chlorpromazine	0	+	14
Na Phenobarbital	7.1	+	14
Diazepam	79.8	$\pm$	14
Na Barbital	57.1	$\pm$	14
Benzpyrene	100.0	—	16
3-Methylcholanthrene	100.0	—	16

Specific binding of 30 nM ^3H -dexamethasone to dissociated cells is total binding minus that in the presence of 10^{-8} M unlabelled dexamethasone²³ as in Fig. 1. Each assay was carried out in triplicate for various drugs and gave specific binding in the presence of 500 nM drug. % control = (Specific binding in presence of drug/specific binding in absence of drug) $\times 100\%$. Control binding; $100\% = 185.1 \pm 15.5$ fmol ^3H -dexamethasone per 10^6 cells.

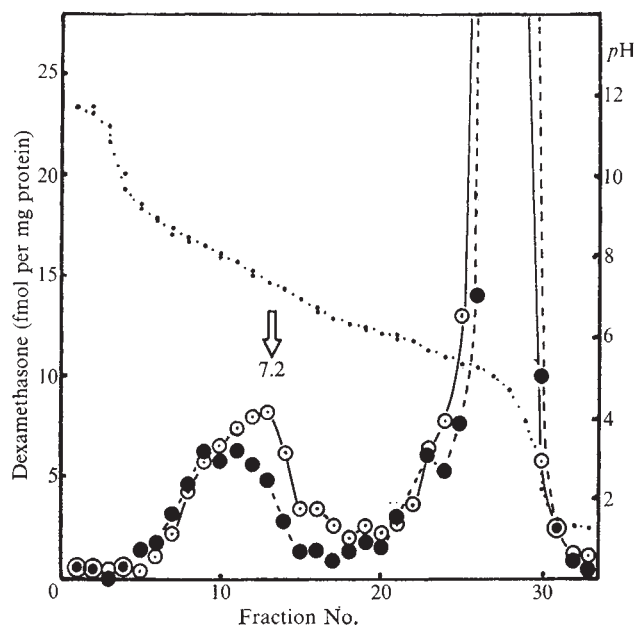


Fig. 3 Electrofocusing of cytosol ^3H -dexamethasone-carrying protein (after treatment with either plain or calf thymus DNA (Sigma)-cellulose)²⁶ at 400 V, for 20 h at 0 °C. Cultured cells were incubated in roller bottles for 40 min with 10 nM ^3H -dexamethasone in BME without foetal bovine serum. Cells were washed of excess ^3H -dexamethasone and dissociated with 0.25% trypsin. The cytosol was prepared by sonication followed by ultracentrifugation at 100,000g for 50 min. The cytosol preparation was treated with either plain or DNA-cellulose for 40 min. The DNA has absorbed the pI 7.2 peak (arrow), thus fulfilling the criterion for a glucocorticoid receptor of uptake by DNA. ●, DNA-cellulose; ○, plain cellulose.

benzpyrene and 3-methylcholanthrene¹⁶. Moreover, a 100-fold concentration of non-radioactive phenytoin included in the incubation medium depressed uptake of 10 nM ^3H -dexamethasone in nuclei isolated by the method of Maggio *et al.*²⁷ (16.3 ± 0.2 compared with 2.8 ± 1.8 fmol per 10^6 cells). Thus, phenytoin prevented nuclear uptake and cell binding of ^3H -dexamethasone.

Our results show the existence of the glucocorticoid receptor with a pI value of 7.2 in the cytosol of cultured human palatal cells. This pI and the K_d value correspond closely with those for murine foetal palatal cytosol binding of cortisol, which is correlated with susceptibility to corticoid-induced cleft palate and is regulated by a gene in or near the *H-2* locus^{5,6}. A receptor mechanism has also been invoked to explain why medroxyprogesterone acetate produces cleft palate in rabbits, but not in rats, since medroxyprogesterone acetate effectively competed with the binding of ^3H -dexamethasone to the rabbit, but not to the rat liver glucocorticoid receptor¹⁷. We have found ^3H -dexamethasone largely in the unmetabolised state in the nuclei of cultured human foetal palatal cells 40 min after its addition to the incubation medium. This suggests nuclear uptake of the glucocorticoid-receptor complex, which presumably may then result in repression or derepression of protein synthesis.

The possibility that human cleft palate may be caused by steroid treatment is controversial¹⁸⁻²¹. Fraser²¹ believes that five cases of cleft palate out of 260 treated with corticoids, or 1.9% reported, is statistically significant, since the anticipated frequency of cleft palate is 1 in 2,500 or 0.04%. He doubts that 12,500 women ($5 \times 2,500$) within the population surveyed would have been receiving corticoids during pregnancy. Serment and Ruf²², however, found only three cases of cleft lips and palates among 428 corticosteroid-treated women, or an incidence of 0.7%. Thus, steroid treatment during pregnancy has a fairly low probability of harming the foetus, but there is a risk that it may induce a cleft in the baby.

The pI of the ^3H -dexamethasone binding protein in human foetal palatal cells in culture is similar to that of the murine ^3H -corticoid binding protein associated with susceptibility to cleft palate⁶. This suggests that the protein in human foetal palates is a candidate for a palatal glucocorticoid receptor possibly mediating cleft palate by virtue of its binding to corticoids and nuclear uptake of the receptor complex with, presumably, activation or depression of transcription. This hypothesis is supported by the present observations that phenytoin, which produces cleft palate in the A/J mouse strain¹⁵, and in the human²³, prevents cellular binding and nuclear uptake of ^3H -dexamethasone and that chlorpromazine and sodium phenobarbital, which are also cleft palate inducers in A/J (ref. 15), are also strong blockers of binding of ^3H -dexamethasone to the human foetal palatal cells. Drugs which have little effect in producing cleft palate^{15,16}, however, either poorly displace or do not displace dexamethasone from the cells. Since binding has been studied in whole cell preparations, it may represent cumulative interactions at the membrane (which may be receptor associated) or at the cytosol receptor followed by nuclear uptake. The teratogens may either prevent entrance of the ^3H -dexamethasone into the cell or they may compete directly at the receptor level. The former explanation is possible because these agents are known to express their central activity by altering thresholds resulting from an interaction with neuronal membranes. The latter possibility is consistent with current thinking on the pharmacological action of hormones and receptors, which suggests that several hormone receptor interactions can be interfered with by drugs that either block the actions of steroid hormones or enhance them²⁴. These compounds may resemble the hormones, as in the case of spironolactones, or may be structurally quite different, such as diethylstilboestrol or phenylbutazone.

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Contribution of a caesium-sensitive conductance increase to the rod photoresponse

THE response of vertebrate photoreceptors is thought to be generated by a light-dependent Na^+ conductance which is relatively high in darkness and decreased by illumination, so that light hyperpolarises the membrane potential towards the potassium equilibrium potential (E_K) (refs 1–3). However, this simple model does not explain many of the complexities of the receptor response. We have carried out experiments in which the retina was superfused with Ringer solution containing caesium chloride and we report here that many of the complexities of the receptor response can be removed reversibly by this treatment. We believe that this is because Cs^+ blocks a conductance increase which is activated by membrane hyperpolarisation, and which is largely responsible for the rapid decay of the receptor potential in bright light.

According to the simple model, receptor input resistance should increase during the light response. Although increases in receptor resistance have sometimes been observed^{1,2}, measurements in rods do not show a consistent increase and have even demonstrated decreases^{4–6}. Also according to the model, the Na^+ conductance should become very small in bright light and, since receptors have little Cl^- permeability⁷, the membrane potential should hyperpolarise to E_K . In fact, the maximum response amplitude is always considerably more positive than E_K (ref. 7), and in steady bright light the amplitude decays from its maximum to an even more positive potential. Finally, the waveform of the receptor response to bright light exhibits a prominent initial hyperpolarising transient, which cannot be readily explained by changes in the single light-dependent conductance postulated by the model⁸.

External Cs^+ has been shown to block inward-going rectification in starfish eggs⁹, and inward potassium currents in the squid axon^{10,11} and at the node of Ranvier¹². In rods superfused with Cs^+ , the waveform of the response is simplified and lacks the initial transient. In addition, a saturating response hyperpolarises the cell very near to E_K , and the response is invariably accompanied by a large increase in input resistance.

Retinae were removed from the eyes of dark-adapted *Bufo*

Fig. 1 Effect of Cs^+ on the waveform of rod photoresponses. Responses in normal and 2 mM CsCl Ringer from a dark-adapted rod have been superimposed at five light intensities. Numbers to the left give intensities in quanta cm^{-2} for 100-ms, 501-nm flashes of diffuse light, of duration as indicated on the uppermost trace. Intensity responses of 7.6 and 8.0 in normal and CsCl Ringers represent averages of four responses. Other traces are single responses. At 7.6 the waveforms are nearly identical, but in brighter light, Cs^+ responses are always larger and lack the fast decay. Dark membrane potential, -35 mV.

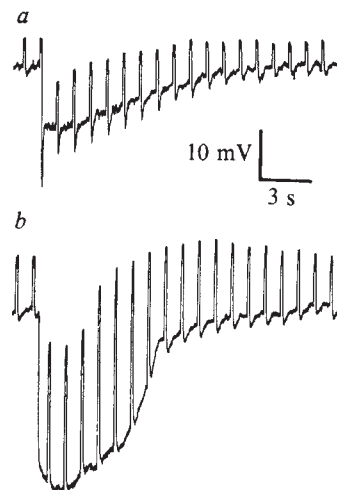
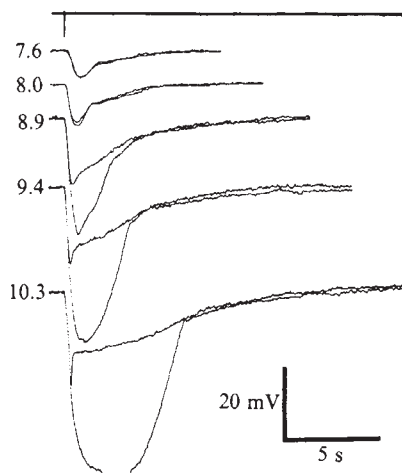


Fig. 2 Light-evoked change in rod input resistance in *a*, normal and *b*, 2 mM CsCl Ringer. Responses are to 109-ms, 516-nm flashes of diffuse light at $11.2 \log$ quanta cm^{-2} per flash. Pulses of constant current (0.1 nA) were injected through a single micropipette using an active bridge circuit. The change in input resistance can be measured directly from these records as the difference in pulse height in the dark and during the light response. Dark membrane potential, -30 mV.

marinus in dim red illumination and were secured with receptors upwards in a perfusion chamber. Oxygenated Ringer flowed from a gravity-controlled perfusion system into a pool above the retina, and intracellular recordings were made from rods as described previously¹³. Normal Ringer had the following composition: 106 mM NaCl , 1.8 mM Na_2SO_4 , 0.13 mM NaHCO_3 , 2.5 mM KCl , 1.2 mM MgCl_2 , 1.8 mM CaCl_2 , 5.6 mM glucose and 3.0 mM HEPES, adjusted to pH 7.8 with approximately 2 mM NaOH . Zero Na^+ Ringer had the same composition as normal Ringer with the following exceptions: Na_2SO_4 and NaHCO_3 were omitted, NaCl was replaced by choline chloride, pH was adjusted with KOH , and KCl was added to bring $[\text{K}^+]_o$ to 2.5 mM. All experiments were carried out at approximately 22 °C.

When the dark-adapted retina was superfused with Ringer to which 2 mM CsCl had been added, there was little or no change in rod dark membrane potential, but the waveform of the rod photoresponse was altered dramatically. Figure 1 shows superimposed responses of a rod in normal and CsCl Ringer at five different light intensities. At dim intensities (for example, $\log I = 7.6$), the responses were almost identical in waveform, but as light intensity increased, the responses diverged, and the differences in waveform were most conspicuous at the brightest, saturating intensity ($\log I = 10.3$). At this intensity, the response in normal Ringer exhibited a fast decay which was absent in CsCl . The maximum amplitude of the response is also much larger in CsCl , increasing from 26 mV in normal Ringer to 51 mV in this cell. The increase in maximum amplitude in going from normal to CsCl Ringer averaged 11 mV for 14 cells, but tended to be larger than this (up to 25 mV) in the cells giving the largest responses.

The largest responses in CsCl Ringer hyperpolarised the cell very near to the potassium equilibrium potential. We demonstrated this by measuring responses to saturating lights in normal and 2 mM CsCl Ringer, and then superfusing the retina with zero Na^+ Ringer containing 2 mM CsCl . Since the photoreceptors have little resting Cs^+ or Cl^- permeability⁷, the membrane potential in CsCl -zero Na^+ Ringer should be very close to E_K . Although our estimate of E_K varied from cell to cell, averaging -79 mV but ranging from -66 to -97 mV, E_K was always within a few mV (1.7 ± 0.8 , mean difference $\pm$ s.e.m., $n = 4$) of the potential reached by the saturating photoresponse in CsCl Ringer. In one typical cell, the membrane

potential was -30 mV in the dark, hyperpolarised during a saturating light flash to -56.3 mV in normal Ringer and to -69.3 mV in CsCl, and then hyperpolarised to -72.5 mV in zero Na^+ . Cs^+ seems to block specifically a component of fast decay which, in normal Ringer, prevents the membrane potential from reaching E_K .

Since Cs^+ seems to block a component of the receptor waveform, we next examined the effect of CsCl on the change in receptor input resistance during the light response. We injected pulses of constant current through a single micropipette using an active bridge circuit while stimulating the retina with broadfield light. In normal Ringer our results were variable. In some cells a saturating light response was accompanied by an increase in input resistance as large as 43 m Ω (Fig. 2), whereas in other cells, the resistance decreased by as much as 27 m Ω , and for six cells the mean change in input resistance for a saturating light flash was -2 ± 5 m Ω (mean $\pm$ s.e.m.). In contrast, large increases in resistance always accompanied the light response in 2 mM CsCl. For the cell in Fig. 2, the resistance increase in the presence of CsCl was 187 m Ω , or 144 m Ω greater than in normal Ringer. We have repeated this experiment with pulses of various amplitudes and both polarities, and with several different stimulus intensities. We have also added Cs^+ to a Ringer containing 1 mM CoCl_2 and 12 mM TEA to minimise any contribution to the resistance measurement from voltage-dependent Ca^{2+} and K^+ conductances¹³. In every case we obtained the same result: the change in input resistance during the light response was greatly increased in CsCl, averaging for all cells studied 86 ± 14 m Ω (mean $\pm$ s.e.m.) for a saturating light intensity.

These experiments show that, in addition to the light-dependent decrease of Na^+ conductance, there is another conductance which normally increases during the light response but is blocked by Cs^+ . This conductance increase cannot be produced by synaptic input on to the rods, since the addition of 2 mM CoCl_2 to the Ringer blocks synaptic transmission

Fig. 3 Effect of Cs^+ on the steady-state current-voltage curve of a rod. The steady-state potential of the rod was recorded with one side of a double-barrelled micropipette ($1\text{--}2$ m Ω coupling resistance), while constant currents of 5 s duration were applied through the adjacent side. The normal current-voltage curve ($\bullet$) has a region of outward-going rectification positive to the dark membrane potential, which is markedly reduced in 12 mM TEA Ringer (see ref. 13). Addition of 2 mM CsCl to the Ringer had no effect on the outward-going rectification but caused an apparent increase in resistance to inward currents ($\circ$).

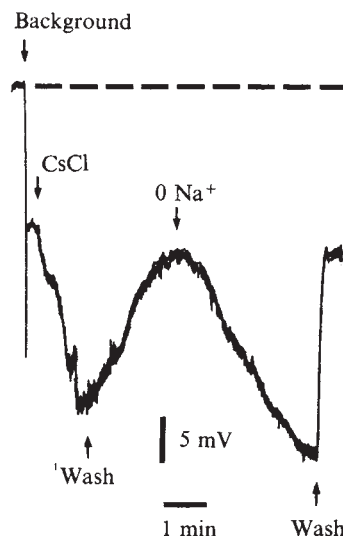
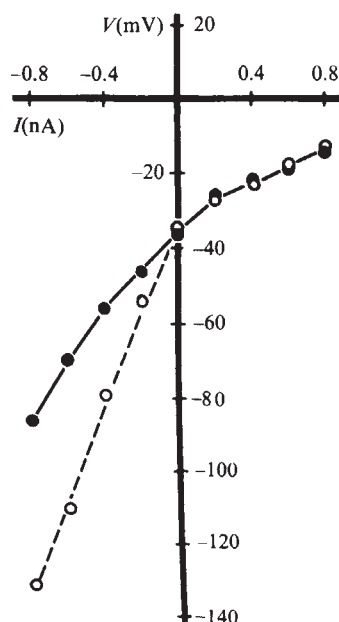


Fig. 4 Effect of Cs^+ and zero Na^+ on rod membrane potential in the presence of bright background light. Initially the retina was dark adapted and in normal Ringer. Arrow in upper left indicates turning on of 501-nm diffuse background light, bright enough to produce incremental saturation (13.5 log quanta $\text{cm}^{-2} \text{s}^{-1}$). The rod responded to this background with a transient hyperpolarisation, which decayed quickly to a steady plateau. In this background no increment responses could be recorded from this cell even at the brightest flash intensities available. The background was on for the period illustrated here, but later it was extinguished and the sensitivity of the rod recovered partially. At the second arrow, the retina was superfused with 2 mM CsCl Ringer; at the third (Wash), it was returned to normal Ringer; at the fourth, it was superfused with zero Na^+ Ringer; and at the fifth, it was returned again to normal Ringer. Dashed line indicates dark membrane potential.

at the receptor synapse¹⁴ but does not eliminate the fast decay of the rods. Furthermore, Cs^+ has the same effect on the rod waveform in Co^{2+} Ringer as in normal Ringer.

Rod current-voltage curves suggest that the Cs^+ -sensitive conductance increase is not produced directly by light, but indirectly by membrane hyperpolarisation. The steady-state current-voltage curves of rods were unaffected by Cs^+ except at hyperpolarising currents (Fig. 3) where Cs^+ greatly increased the amplitude of the voltage drop produced by the current. This suggests that at hyperpolarising potentials, the steady-state conductance of the rod is much greater in normal than in Cs^+ Ringer. This result is consistent with our view that Cs^+ blocks a conductance increase which is activated by membrane hyperpolarisation. It is not possible, however, to make detailed inferences about the potential dependence of the conductance increase from Fig. 3, since the action of Cs^+ may itself depend on membrane potential⁹.

If a conductance increase produces the fast decay of the receptor response to bright light, then the reversal potential for this conductance must be much more positive than E_K . The results of the experiment shown in Fig. 4 suggest that the fast decay may be produced at least in part by an influx of Na^+ . In this experiment we first illuminated the retina in normal Ringer with a steady background light ($\log I = 13.5$) which was more than bright enough to produce increment saturation¹⁵. In these conditions even the brightest flash produced no detectable response, and extracellular recordings indicated that there was no dark current entering the rod outer segment^{16,17}. After approximately 20 s, 2 mM CsCl was added to the Ringer and the rod hyperpolarised, indicating that the Cs^+ -sensitive conductance was high, probably as a result of the steady hyperpolarisation of the cell in the presence of the background light. Returning the retina to normal Ringer brought the potential back up approximately to the value observed before the introduction of Cs^+ . The retina was then superfused with zero Na^+ Ringer (without Cs^+), and the rod

hyperpolarised, presumably to E_K . The permeability of the rod to Na^+ in these conditions is probably not the result of the light-dependent Na^+ conductance, since in the presence of the background there is no dark current, and the light-dependent channels are presumably closed. We propose that Na^+ passes through the Cs^+ -sensitive conductance.

The intracellular responses of the rods in the presence of Cs^+ are similar in waveform to the photocurrents recorded extracellularly from rod outer segments¹⁷. A detailed investigation of receptor responses in CsCl Ringer will, we hope, reveal the role of the Cs^+ -sensitive conductance in the normal physiology of the rods.

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Leaky UAG termination codon in tobacco mosaic virus RNA

THE RNA of tobacco mosaic virus (TMV) can be translated in various cell-free systems^{1–3} and in amphibian oocytes⁴, to yield two large polypeptides of molecular weights (MW) approximately 110,000 (110K) and 160,000 (160K). Products of the same sizes are found in infected plants^{2,5} and protoplasts⁶ and they are probably components of the viral replicase^{7,8}. I show here that synthesis of these two proteins is initiated at the same site; the larger product is generated by partial readthrough of an amber (UAG) termination codon, and in the presence of yeast amber suppressor tRNA, the *in vitro* synthesis of the 160K protein is increased whereas the yield of the 110K protein is diminished.

TMV RNA is not long enough to contain totally independent cistrons for two products of MW 110,000 and 160,000. (The smaller product migrates slightly faster than β -galactosidase on SDS-polyacrylamide gels, and was previously designated 130,000 or 140,000 (refs 1–4); the revised estimate follows the determination of the molecular weight of β -galactosidase as

116,000 by amino acid sequencing⁹.) The coding sequences for these large proteins must therefore overlap in some way. The nature of this overlap was examined by translating TMV RNA in an mRNA-dependent reticulocyte lysate³ in the presence of ³⁵S-methionine, separating the products on acrylamide gels, digesting them with trypsin, and analysing the labelled peptides. The patterns obtained from the 110K and 160K bands were very similar (Fig. 1a) except for the presence of extra peptides in the larger protein. Thus the two polypeptides are translated in the same phase, and probably overlap throughout the length of the smaller one.

When this experiment was repeated using formyl-³⁵S-Met-tRNA_i in place of ³⁵S-methionine, the labelled peptides derived from the two proteins were similar (Fig. 1b), showing that they have the same N-terminal sequence, and therefore the same initiation site on the RNA. This result was somewhat unexpected, since, as mentioned previously¹⁰, TMV RNA binds two ribosomes in an initiation assay (in which translation is blocked with sparsomycin). Two different methionine-containing dipeptides are formed in these conditions, neither corresponding to that obtained in a similar experiment with the subgenomic coat protein mRNA (ref. 8). In view of the data presented here, it seems likely that one ribosome binds to the common initiation site for the 110K and 160K proteins, and the other binds elsewhere, perhaps to the initiation site for the 30,000-MW TMV protein¹. This is normally made from a subgenomic mRNA, but small quantities are always found when (initially) full-size viral RNA is translated in the wheat germ^{1,2} or reticulocyte³ systems. Alternatively, the ribosomes might bind to adjacent sites at the 5' end of the RNA, giving rise to two pairs of 110K and 160K products which would not be resolvable by gel electrophoresis. This could explain the presence of multiple N-terminal peptides in these proteins (Fig. 1b), although this may just be an artefact of the digestion conditions used, since the proteins were digested in gel fragments that had been dried and re-swollen, and no precautions were taken against partial oxidation.

The 110K and 160K proteins have a common N terminus, so they must differ at the C-terminal end. The kinetics of their appearance *in vitro*, and their stability during prolonged chases with unlabelled amino acids *in vitro* (my unpublished work) and *in vivo*¹¹, are inconsistent with the idea that the smaller protein arises by proteolytic cleavage of the larger one. An alternative explanation is that the larger product is produced by readthrough of a termination codon at the end of the sequence coding for the 110K protein, in which case the yield of the 160K product should be increased by the addition of an appropriate suppressor tRNA¹². Figure 2a shows the products of translation assays carried out in the presence of partially purified amber (UAG) and ochre (UAA) suppressor tRNAs from appropriate strains of yeast. Amber suppressor tRNA increased the amount of readthrough from less than 10% to nearly 70% (defined as: (radioactivity in the 160K band $\times$ 100/radioactivity in both bands)). Ochre suppressor tRNA had a smaller, but nevertheless significant effect, whereas wild-type yeast tRNA had none. Mouse liver tRNA, which stimulates translation of TMV RNA about fourfold in this system³, did not alter the relative yields of the two products, nor did it interfere with the suppression by yeast tRNA. Readthrough, however, was increased to almost 40% by supraoptimal concentrations of Mg²⁺ (Fig. 2b), a result similar to the Mg-induced suppression of an amber mutant of bacteriophage R17 in an *E. coli* cell-free system¹³. Addition of MgCl₂ at various times after inhibition of initiation showed that it was only required when the ribosomes reached the end of the 110K cistron (Fig. 3), consistent with a direct effect on termination at this point. Control experiments with base-hydrolysed suppressor tRNA showed that its activity was not due to contaminating Mg²⁺ (R. F. Gesteland, personal communication).

These results suggest that the cistron for the 110K protein terminates with a UAG codon, which, even in the absence of suppressor tRNA is subject to a significant degree of read-

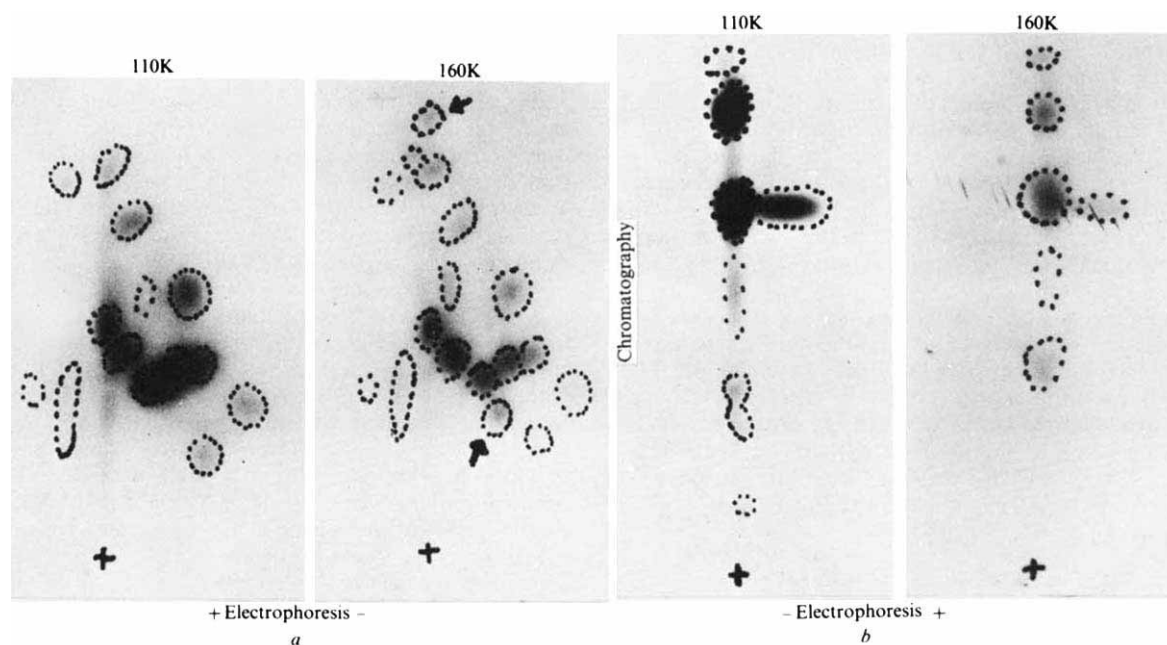
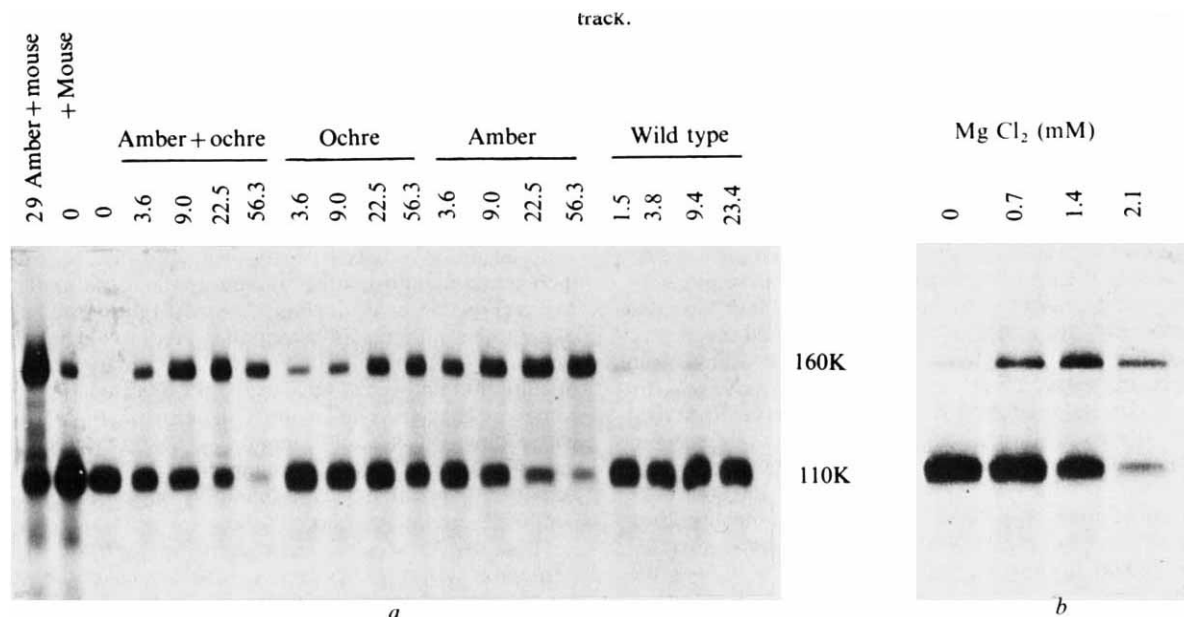


Fig. 1 Peptide analysis of translation products. TMV *vulgare* was disrupted with SDS and intact RNA purified by sucrose gradient centrifugation. RNA (20 μ g) was added to 200 μ l of micrococcal nuclease-treated reticulocyte lysate³ containing 60 μ g ml⁻¹ mouse liver tRNA and 250 μ Ci ml⁻¹ ³⁵S-methionine. After incubation for 1 h at 30 °C, the incubation was loaded on to a 12.5% SDS-polyacrylamide gel³ and electrophoresed at 50 V overnight. Bands were located by autoradiography of the dried gel, excised, cut into small pieces and incubated overnight at 37 °C in 4 ml 50 mM NH₄HCO₃, 50 μ g ml⁻¹ TPCCK-treated trypsin. The supernatant was removed, and fresh trypsin added. After a further 4 h the supernatants were pooled, lyophilised, and passed through Sephadex G-25 to remove acrylamide and salts. The larger and smaller ³⁵S-methionine labelled peptides were pooled separately at this stage and lyophilised. Peptides were electrophoresed in 5% acetic acid, 0.5% pyridine, pH 3.5 on 10 $\times$ 10 cm silica thin-layer plates at 500 V for 15 min. They were then chromatographed in butanol-pyridine-acetic acid-water (15:10:3:12) at right angles to the first dimension. Formyl-³⁵S-Met-tRNA_f was prepared from reticulocyte tRNA as described by Stanley¹⁷ and purified on an RPC-5 column¹⁸. It was added to the translation mix together with 1 mM methionine, and the products were treated as described above. Autoradiographs exposed for 1 week (a) and 1 month (b) are shown. The peptides have been outlined because they are not all clearly visible after reproduction. The crosses mark the origins. a, Analysis of the ³⁵S-methionine-labelled products. Only the smaller peptides are shown; arrows mark 160K-specific peptides; b, analysis of the formyl-³⁵S-Met-tRNA_f-labelled products.

through. An analogous prokaryotic example is the leaky UGA terminator for the coat protein of Q β (ref. 14). There are several indications that readthrough of the TMV termination codon occurs particularly easily. First, since the 160K product

is made in tobacco cells, wheat germ extracts and *Xenopus* oocytes, as well as in rabbit reticulocyte lysates, the read-through of this codon must be widespread in eukaryotic cells in spite of their different tRNA complements. Second, although

Fig. 2 Suppression of the 110K termination codon. a, TMV RNA (10 μ g ml⁻¹) was translated in the reticulocytesystem in the presence of ³⁵S-methionine and the products analysed by SDS gel electrophoresis (10% acrylamide). Portions of a fluorogram¹⁹ are shown. tRNA from mouse liver (160 μ g ml⁻¹) or from wild-type, amber and ochre strains of yeast was added to the incubations as indicated. Yeast tRNA concentrations are given in μ g ml⁻¹; for incubations containing both suppressors, the indicated amount of each was added. b, TMV RNA was added at 100 μ g ml⁻¹, mouse liver tRNA at 60 μ g ml⁻¹, and MgCl₂ at the concentrations indicated (mM). The reticulocyte extract itself contributed about 1 mM Mg²⁺; for normal translation, an extra 0.5 mM was added. Equal volumes of incubation were loaded on each



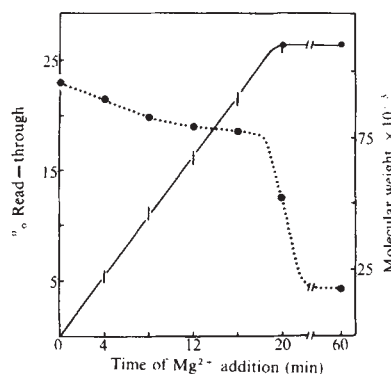


Fig. 3 Effect of delayed addition of Mg^{2+} on readthrough of the 110K termination codon. An incubation was set up as in Fig. 2b, with no added $MgCl_2$. Initiation was inhibited after 2 min by addition of edeine ($4 \mu M$). At the indicated times 10- μl aliquots were withdrawn, added to 1 μl of 25 mM $MgCl_2$, and incubated in parallel for a total of 60 min. Samples of these were analysed on an SDS gel. The 110K and 160K bands were located by fluorography, excised and dissolved in 30% H_2O_2 at $90^\circ C$, and counted in aqueous scintillant. Percentage readthrough (.....) was calculated after background subtraction. Typical c.p.m. were 110K: 26,000; 160K: 7,300; background ($> 160K$): 1,500. The progress of the ribosomes along the RNA was monitored by removing samples of the original incubation at intervals into SDS-containing buffer. The size of the nascent protein (vertical bars) was estimated by gel electrophoresis of these samples. Note that elongation was slowed by the sub-optimal Mg^{2+} concentration: about half the ribosomes had completed the 110K protein after 20 min.

elevated Mg^{2+} increases readthrough of this termination codon in the reticulocyte system, no 'suppression' of a Q β amber mutant by Mg^{2+} could be detected in these conditions (my unpublished results). In addition, although the ability of the ochre suppressor tRNA to enhance readthrough of this UAG codon is consistent with the 'wobble hypothesis'¹⁵, it is significant that ochre strains of yeast do not normally suppress amber mutations¹⁶, although this ochre suppressor tRNA did have some activity in the reticulocyte translation system programmed with Q β^{am} RNA.

The extent of readthrough of the TMV amber codon *in vitro* at moderate Mg^{2+} concentrations seems to be sufficient to account for the observed synthesis of 160K protein *in vivo*⁶, although more complex mechanisms cannot be excluded. It thus seems that the RNA sequence around a termination codon can reduce the efficiency of termination at that site in normal eukaryotic cells; TMV makes use of this phenomenon to produce different amounts of two proteins from a single initiation site. A similar mechanism has been suggested for the synthesis of the reverse transcriptase of avian sarcoma and murine leukaemia viruses, by readthrough from the *gag* gene into the *pol* gene²⁰⁻²². Preliminary (unpublished) results with Rous sarcoma virus indicate, however, that although amber suppressor tRNA allows the synthesis *in vitro* of a slightly larger protein than Pr 76 (the *gag* gene product), it does not increase the yield of the 180,000-MW putative *gag-pol* precursor. This suggests that a more complex mechanism, such as frameshift suppression, is involved, or that the 180,000-MW protein and Pr 76 are made from different mRNA species.

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Determination of plasmid molecular weights from ultraviolet sensitivities

MECHANISMS for the repair of ultraviolet (200-300 nm) irradiated DNA have been well studied in connection with cells and bacteriophages¹, but not with plasmids. During investigations of plasmid ultraviolet (UV) sensitivity by an *Escherichia coli* transformation assay², we found that the principal *E. coli* repair systems act on UV-irradiated plasmid DNA, exerting a major effect on plasmid survival. In host cells lacking these repair functions, however, the UV survival of transforming activity depends essentially on plasmid size alone, and decreases with increasing molecular weight. This gives an accurate and convenient method for determining plasmid size. In addition, the lower UV sensitivity of small plasmids in such conditions provides an effective way to select for mini-plasmids.

Both excision and recombination repair act on plasmid DNA which has been UV irradiated extracellularly before uptake: UV survivals of transforming activity for the plasmid R6K on *recA*⁺ *uvrA*⁺, *recA*⁺ *uvrA*⁻, and *recA*⁻ *uvrA*⁻ hosts show progressively greater sensitivity with decreasing cellular repair functions. Plasmids irradiated intracellularly, and then extracted and tested for transforming activity, manifest increased activity with incubation time before extraction from a *uvr*⁺ or *rec*⁺ cell, whereas those from a *uvr*⁻ *rec*⁻ cell do not. This shows that plasmids irradiated intracellularly are also repaired by the *REC*⁺ and *UVR*⁺ pathways. Separate experiments have shown that photoenzymatic repair of extracellularly irradiated R6K DNA, after uptake by competent *E. coli* cells, likewise occurs efficiently, with a maximum dose modification factor of about 0.5 in a *uvrA*⁻ strain. Such *in vivo* photoreactivation of a transforming DNA has not been observed before, because the classical transformable species—*Diplococcus pneumoniae*, *Haemophilus influenzae*, and *Bacillus subtilis*—do not possess photoreactivating enzyme.

In the absence of all host cell repair, one might expect that the exponential UV survival curves would depend solely on the number of lethal photoproducts (mostly cyclobutadipyrimidines or 'pyrimidine dimers') generated initially in the plasmid by ultraviolet. In fact, the F_{37} of R6K (that is, the UV influence, $2.2 J m^{-2}$, which reduces its transforming activity by a factor of e^{-1} , or 0.37, in a totally repair-deficient host) is precisely that required to generate an average of one 'pyrimidine dimer' in a DNA molecule of that size and base composition, as judged from photochemical data³. Comparison of transforming activity survival for plasmids of different sizes in this same host shows that the F_{37} is, in fact, inversely proportional to the molecular size, as it should be if a single unrepaired damage site on the plasmid DNA is sufficient to inactivate. When the F_{37} s for these plasmids are plotted as a function of inverse molecular weights (Fig. 1) a single calibration line is obtained, from which the molecular weight of any plasmid with DNA of similar base composition can be determined. To test the sensitivity of the method, we also

examined a series of deletion and insertion mutants of R6K, isolated and characterised by R. C. Clowes and coworkers (see Fig. 1b). The accuracy with which an F_{37} can be determined depends on the reproducibility of the transformation experiments, and on the UV survival level at which experiments can be conducted. At survival levels of about 10^{-4} , a 10% difference in F_{37} would correspond to a twofold difference in surviving numbers, and is easily determined. The difference between plas-

mids pPH11 (18.6 MD) and pRC340 (18.5 MD) is too small for consistent measurement, although three independent experiments all indicated slightly higher UV resistance for pRC340, as would be expected because of its slightly smaller size.

These facts also suggest a simple technique for isolating mini-plasmids. When a heterogeneous plasmid population is subjected to UV irradiation, the larger molecular species will accumulate more photochemical damage than the smaller. Consequently when the treated DNA is used to transform repair-deficient cells, any mini-plasmids present should be found with enhanced frequency among the transformants. By this means we have easily isolated pure, monomeric pMB9 from a plasmid DNA preparation containing only 0.1% monomers.

It is possible that other methods for producing mini-plasmids have actually functioned by this same principle. For example, the widely used plasmid pSC101 (ref. 5), supposedly produced from a sheared R6 fragment which recircularised intracellularly after uptake⁶, might well have been an already existing circular plasmid selected by preferential fracture of the higher molecular weight (normal sized) species in the preparation. Similar selection should be provided by chemical inactivation.

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Corrigenda

In the article 'Can a myosin molecule bind to two actin filaments?' by G. Offer & A. Elliott, *Nature* **271**, 325, in paragraph 2 on page 327, lines 15-16 should read . . . then 2 is rotated 120° anticlockwise with respect to 1 . . . (Not clockwise).

In the letter 'Toxicity of diphtheria toxin for lymphoblastoid cells is increased by conjugation to antilymphocyte globulin' by P. E. Thorpe *et al.*, *Nature* **271**, 752, in Fig. 2, (a) represents results from 1-h incubation (not 24-h) and (b) represents values from 24-h incubation.

Erratum

In the letter 'Evolution of dynamical systems with time-varying gravity' by A. Marchant and V. Mansfield, *Nature* **270**, 700, the last line on page 699 should read . . . (such as galaxies) cannot be used to place limits on G because, as . . . (An overdot was omitted).

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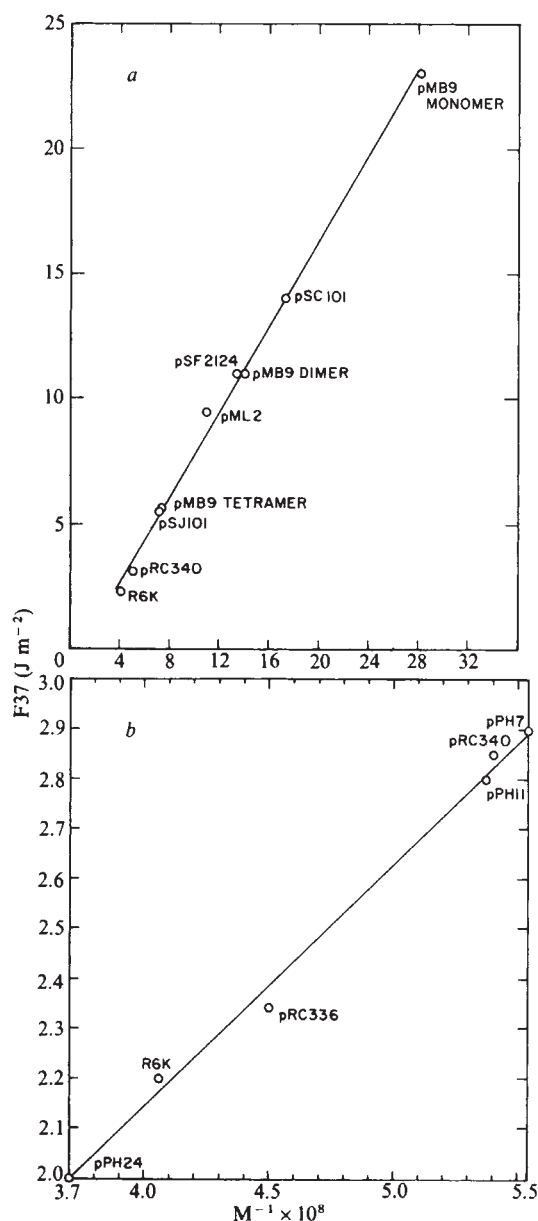


Fig. 1 Relationship between ultraviolet sensitivity of plasmid DNA-transforming activity and plasmid molecular weight. *a*, Wide size range (3.5-24 megadaltons); *b*, narrower size range (18.1-27 megadaltons). Each plasmid indicated, irradiated with several 254-nm UV fluences, was used to transform *E. coli* CSR603 (a *uvrA6 recA1 phr* strain derived from AB1886 (ref. 4)) to a drug-resistance character carried by the plasmid: tetracycline resistance for pMB9 and pSC101; kanamycin resistance for pSJ101 and pML2; and ampicillin resistance for pSF2124, pRC340, pPH7, pPH11, pPH24, pRC336, and R6K. The fluence required to reduce transforming activity to 37% of the value observed with unirradiated plasmid was plotted against the reciprocal of the molecular weight in daltons. (Abscissa scale values were multiplied by 10^8 before plotting.) Approximate transforming frequencies in the various experiments were: $10^6 \mu\text{g}^{-1}$ DNA for pMB9 and pSC101; 5×10^4 - $7 \times 10^4 \mu\text{g}^{-1}$ for pMB9 dimer, pSF2124 and pML2; and 2×10^4 - $4 \times 10^4 \mu\text{g}^{-1}$ for pMB9 tetramer, pSJ101, and R6K and its deletion and insertion derivatives (pPH7, pPH11, pPH24, pRC336 and pRC340).

reviews

Phylogenetic problems

E. J. W. Barrington

Origin and Early Evolution of Animals. By Earl D. Hanson. Pp. ix+669. (Pitman: London, 1977.) £23.

PROFESSOR HANSON brings to fruition in this volume his long-standing interest in problems of animal phylogeny. To do so needs some courage, for many zoologists are likely to sympathise with Hyman's view that "such phylogenetic questions as the origin of the Metazoa from the Protozoa" are "insoluble on present information . . . Anything said on these questions lies in the realm of fantasy." Hanson, however, is concerned with evolution at the lower taxonomic levels as well as with the origins of major groups, and he makes a good case for examining all of these matters in depth.

He rightly insists that a primary requirement is that any proposed scheme must be acceptable in terms of natural selection. But, given this overriding condition, evolutionary phylogeny can lead us to interpret evolutionary innovations "not just as morphological or physiological facts, but as selectively advantageous endowments available to all descendants of that line." Phylogeny thus becomes an analysis of evolutionary potential, thereby enlarging our understanding of the principles underlying the diversification of animal organisation.

He begins by dealing with concepts and methodology, briefly reviewing the evolutionary significance of populations and the gene pool, and emphasising the distinction between anagenesis and cladogenesis. From this he draws the conclusion that although the two sets of potentials cannot be sharply separated, it is predominantly the anagenetic groups that will provide the best keys to phylogenetic analysis. He mentions the Chordata as an obvious example, although he deals only with lower forms, from Protozoa to Turbellaria.

He then reviews what he calls the "informational content of organisms". His aim here is to establish what are the "compositional units" of structure and function, how these units are inter-related, and how many different sets of interrelationships are actually found. In other words, how diverse may be a particular structure or function? This part of his treatment is decidedly uneven. Within the 44 pages devoted to it, he seeks answers to his questions at several levels: molecular, cellular, multicellular organisation, and population. Not sur-

prisingly, some of the generalisations with which he emerges do not advance us very far. It is not really helpful to be told that "the brain . . . is made up of various types of nerve cells and contributes a co-ordinatory and information storage and retrieval service", and to suggest that "desert animals excrete almost no fluids but aquatic organisms have the opposite problem of pumping out excess fluids", is, to say the least, an underestimate of the achievements of natural selection, to which he elsewhere attaches such importance.

Much more valuable is his review of the procedural steps needed for determining phylogenetic relationships within a group. Using the concept of the seme as a unit of phylogenetic information, and distinguishing conservative ones (plesio-semes) and innovative ones (aposemes), he aims at a comparison of semes in different species, using where necessary the concept of homology according to the criteria of Remane. This should lead to the identification of a plesiomorph, which is the species, or its nearest representative, containing the largest number of plesio-semes. From here it should then be possible to establish the ancestral form from which a given monophyletic group arose.

There is, of course, much more to the argument than this, and zoologists will find it worthwhile to explore the detail with which Hanson defines and justifies his methodology. The greater part of the volume is devoted to his analysis of its results. He correctly recognises that it is study of lower taxa rather than of higher ones that will lead to the more convincing conclusions, and his treatment of protozoan phylogeny (which, as befits his own special interests, is pursued at length) is

in accord with this. His analysis of aposemic change within individual groups is informative, and, as he points out, the phylogenies suggested by it could be the basis for much further specialist research. But when it comes to discussing the origins of these groups we are left little the wiser. The origin of the Acantharia, for example, "is unclear", and the derivation of ciliates from their presumed zooflagellate ancestors presents "a paradoxical problem".

The reason given for this paradox is instructive. Essentially, it is that the selective advantage of ciliate organisation, compared with that of flagellates, has resulted in the disappearance of the intermediate forms. But this is surely the crucial difficulty in handling major phylogenetic problems. It appears with no less force in Hanson's review of the problem of the origin of metazoans, where again he has to comment on the amount of evolutionary history that must have intervened between the presumed zooflagellate ancestors and the earliest cnidarians.

We are surely forced to conclude that when we confront major phylogenetic issues we must expect to find that the secrets of natural selection are concealed from our gaze as an inevitable result of its own actions. Hyman, then, was right, and it is only too easy to enter realms of phylogenetic fantasy. But Professor Hanson is careful not to do this, and there is much benefit to be gained from his scholarly exposition of the constraints which we must accept if we venture on phylogenetic analysis. □

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Osmoregulation in land arthropods

Water Balance in Land Arthropods. By E. B. Edney. Pp. xii+282. (Springer: Berlin, Heidelberg and New York, 1977.) DM78; \$34.40.

WITHOUT any doubt, the survival of the smaller scale terrestrial animal depends on its ability to maintain adequate internal water, and almost every structure and system in an insect appears either adapted to water conservation or compromised by that need. Apart from intrinsic interest in the physical and physiological mechanisms

involved, the subject of Professor Edney's book is therefore fundamental to understanding so much about these animals of great ecological and applied importance.

The virtue of the work is that it reviews most comprehensively, and it reports accurately in clear and simple style, the extensive literature on cuticular permeability in relation to structure, evaporative loss, water uptake, and the phenomena associated with respiration, osmoregulation and excretion, and with feeding. There is a

chapter on the special problems of the insect egg, and just sufficient on the physics of evaporation and the physical chemistry of water transport to point the pitfalls over which serious errors have been made. Professor Edney has achieved a good balance between the representation of insects and the other land arthropods; he has integrated his own considerable research contribution without undue prominence or modesty, and has given proper credit to discovery regardless of its date. This is a valuable reference book, particularly for the tabulation of facts from a scattered literature. Having set out the various theories under each main heading, the author is content to cast his vote in favour of those at present most convincing, and to indicate in a coda to each chapter where the larger gaps in our knowledge remain.

If there is a disappointment, it lies in finding that the chapter headed "Conclusions" is but a summary of the preceding text, in which water loss and

uptake has been considered system by system. But many physiological systems other than those selected for special study in the book, change significantly with the animal's state of hydration; it is a remarkable feature of the insect that it can tolerate huge fluctuations in internal water. Its behaviour changes and its response to its environment is mediated through its humidity receptors. Some of these matters are mentioned briefly in the general text, but one feels that if the book had ended with an essay integrating the material into a consideration of the whole organism, what is unquestionably a valuable review without current rival, would have become an outstanding biological treatise.

James Beament

James Beament is Head of the Department of Applied Biology at the University of Cambridge, and Chairman of the Natural Environment Research Council, UK.

Gas-phase energy transfer

Vibrational and Rotational Relaxation in Gases. By J. D. Lambert. Pp. 142. (Oxford University: Oxford, London and New York, 1977.) £8.75.

PHYSICAL CHEMISTS and molecular physicists have studied how energy is transferred in intermolecular collisions for many years. In the past decade, this interest has been intensified by the development of a variety of molecular gas lasers, whose operation depends on processes of vibrational and, to a lesser extent, rotational energy transfer between the species in the laser media. Not only has the development of such lasers provided an incentive for much basic research, but also the lasers themselves have provided the means to carry out some of this research, through their ability to excite molecules to specific energy levels for direct studies of 'state-selected' processes.

Although the topics of vibrational and rotational relaxation have been reviewed frequently during the past ten years, all the books on these subjects precede the laser era. Some of them, especially Herzfeld and Litovitz's classic, *Absorption and Dispersion of Ultrasonic Waves*, will continue to be valuable, but this is an appropriate time for a new survey of the field, especially as the application of fixed frequency lasers to the experimental study of energy transfer has reached maturity, whereas only very limited use has been

made, so far, of tunable coherent sources

Few people are better fitted to provide a comprehensive review of gas-phase energy transfer than Dr Lambert. In this monograph, he sets out to provide a clear and concise account of the current state of experimentally derived knowledge. Although the book could be read profitably, and without undue difficulty, by advanced undergraduates, it is unlikely to find a large market here, as few universities offer courses in this subject. On the other hand, it does provide an excellent introduction for postgraduate students starting on research in energy transfer, and it will also be read with benefit and enjoyment by more senior researchers.

The introductory chapters give a simple discussion of molecular collisions and review the major experimental techniques. The three main chapters of the book cover vibrational-translational, vibrational-vibrational, and rotational-translational energy transfer. Here the author demonstrates a sure grasp of his subject. A number of systems are chosen for special consideration. Theory is not emphasised, but the systems are carefully selected, and discussed in sufficient detail to bring out clearly those factors, in the intermolecular potential and collision dynamics, that are chiefly responsible for the observed rates of energy transfer. Overall, this book provides a lucid account of an active field of research and it will be widely read.

Ian W. M. Smith

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Seed pathology

Seed Pathology. By Paul Neergaard. Vols 1 and 2. Pp. 1187. (Macmillan: London, 1977.) £60.

THIS is a valuable and comprehensive contribution which will have wide application as a reference work and practical guide for those concerned with seed pathology, especially those with a direct interest in seed health, disease control measures and seed health testing procedures.

Paul Neergaard is an internationally recognised authority and in this book he has brought together detailed information on seed-borne pathogens, their mode of infection and transmission, and on the subject of their control. Clearly, in writing this book the author had in mind the requirements of workers and teachers in developing as well as developed countries.

It is a pity that the book could not have been organised as a single volume, especially as volume 2 comprises only the glossary, references and index. These extend to no less than 347 pages. The value of the book would not have been seriously impaired with a less extensive glossary and index, and the possible omission of some of the less important references and excessive detail found in certain parts of volume 1.

Volume 1 has been organised in five parts. Part 1 (Pathogens—Diseases—Hosts), which comprises 10 chapters, is a compendium of seed-borne pathogens (including fungi, bacteria, viruses and nematodes) and other economic seed disorders, their distribution and reviews of their harmful effects. Other chapters deal with seed development and seed structure in relation to infection.

Part 2 provides extensive and detailed information on mechanisms of transmission, infection and spread, and on such aspects as the effects of environment on transmission, time of infection, entry and infection of seed parts, systemic infection, pathogenic specialisation, inoculum potential and epidemiological implications of host susceptibility.

The remaining three parts are concerned with the principles of seed-borne disease control, with details of control measures and procedures, and details of equipment notably for chemical seed treatment and seed health testing. The subjects of seed quarantine, tolerance levels and the forecasting of losses from seed-borne diseases are also discussed.

D. J. Griffiths

D. J. Griffiths is Deputy Director of the Welsh Plant Breeding Station, Aberystwyth, Wales.

Hormone action in plants

Hormone Action in the Whole Life of Plants. By K. V. Thimann. Pp.448. (University of Massachusetts Press: Amherst, Massachusetts, 1977.)

THE biochemical and molecular biological approach to physiological questions has gained such ascendancy in recent years that it is rare indeed to find an author attempting a comprehensive, holistic treatment of a topic such as hormone action in plants. When the author is one who can trace his own commitment back to the beginnings of auxin studies and who has since contributed substantially to every aspect of the study of hormones, the book becomes an obvious must for every developmental botanist. Based on a course of lectures presented in 1974 at the University of Amherst while the author was a Visiting Professor, the book is a highly personalised account of the role of hormones in all phases of plant development. The book is not over-scholarly, the treatment being more conversational, even, in places, anecdotal; but it captures in a most expressive manner the fervent nature of scientific research and the latent excitement which underlies it. It is a book to read, not merely to consult, and in doing so, one is made vividly aware that science is not just a collection of facts and figures, but is a product of human creativity, with the creators themselves often being at least as interesting as the results they obtain.

The book follows a logical sequence through the life cycle of the plant, with eleven chapters covering seed germination, cell enlargement and growth, polarity, geotropism, phototropism, leaf and root development, differentiation (particularly in tissue culture), apical dominance, flowering and fruiting, and senescence. Chapters are also included on chemical aspects of the hormones, and on concepts of their mechanism of action. The real strength of the coverage lies in its integrative quality with particular emphasis being placed on the interactions between the various hormones in the regulation of the specific phases of plant development. The most successful chapters are those dealing with the complex multi-component phenomena such as germination, leaf and root development, apical dominance, and fruiting and abscission, all areas of study in which integration of concepts is sorely needed.

There are, however, certain shortcomings to the book. As the book seems to be based on a verbatim record of the lectures, the overall information content is rather low; and the material

that is presented is rather selective, as it is largely based on the admittedly excellent work of the author and his colleagues. Some of this work stretches back to the 1930s and it is remarkable to see how relevant the discoveries of almost half a century ago are today. On the other hand, very recent developments are only lightly touched upon; for example, I could find no mention of hormone receptor proteins.

There are some quite important errors. For example, Fig. 14.11 is stated in the legend to represent a concentration plot of the effect of auxin on glucan synthetase activity in pea stems, whereas the actual figure seems to be a time course. The legend is corrected in the Errata list supplied, but the figure is still at variance with the text on page 414. Although many of the

typographical errors are obvious and present no problem to the intelligent reader, some, such as the one cited, are serious and could confuse the uninitiated, in spite of the comprehensive Errata list.

These points, together with the minimal bibliographic listings, suggest to me that the book should not be recommended as a student text, even though the original lectures were presented to a student audience. It is more a book to be enjoyed by those who already have a sound background in plant physiology. Such readers would, I guarantee, be substantially broadened by the experience.

Harry Smith

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Progress in nitrogen fixation

Recent Developments in Nitrogen Fixation. Edited by W. Newton, J. R. Postgate and C. Rodriguez-Barrueco. Pp. 622 (Academic: London and New York, 1977.) £16; \$31.25.

THE enormous increase of interest in biological nitrogen fixation over the past few years has stimulated the holding of many meetings throughout the world, most of which have resulted in the publication of a symposium proceedings. This has had the effect of flooding the market with such books and compelling the contributors to such meetings to produce appropriate reviews. For many people this has meant the writing of two or more review chapters a year for the past few years. Therefore, one might reasonably ask what yet another collection of such papers has to offer.

In 1974 the Charles F. Kettering Research Laboratory and Washington State University sponsored the First International Symposium on Nitrogen Fixation, with the intention of bringing together scientists from the different disciplines involved in nitrogen fixation research. This facilitated an exchange of ideas between chemists, biochemists, microbiologists, geneticists and others involved in fertiliser technology and nitrogen utilisation. The Proceedings were published in two volumes, edited by W. E. Newton and C. J. Nyman (Washington State University Press: Pullman, 1976). The Second International Symposium on nitrogen fixation was held in Salamanca, Spain in 1976 and the book being considered here is effectively the

proceedings of this meeting, which covered much the same ground as the first Symposium. The papers in this book therefore provide a convenient yardstick for assessing progress over the intervening two years.

How valuable the coverage of the present book is can be surmised from the range of papers and reviews: seven on chemical aspects of nitrogen reduction, five on biochemical and physiological aspects of nitrogenase, thirteen on the physiology and genetics of free-living bacteria and *Rhizobium*, eight on non-legume associations and symbioses, and two on energetic relationships. There are excellent reviews within each area mentioned, as well as a scattering of rather inferior papers that are neither good reviews nor truly original research publications. However the latter are inevitable when all contributors to a meeting have been required to produce manuscripts; they are more than compensated for by the quality of the many other reviews, especially those by the chemically and biochemically orientated authors.

I feel that this book provides a good background analysis of most aspects of nitrogen fixation and that most authors have made some attempt to write reviews that should be intelligible to interested readers from different disciplines. It is a pity that it has taken fifteen months to be published, as this inevitably 'dates' some of the material; it does not, however, seriously affect its value as a reference work and introduction to this fascinating field of research.

John E. Beringer

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obituary

W. W. Pigman

PROFESSOR W. W. PIGMAN, former Chairman of the Department of Biochemistry at New York Medical College died on 30 September 1977, aged 67.

Ward Pigman emerged through the adversities of the Depression years and a career encompassing governmental, industrial, and academic research, as one of the most broadly based and well recognised authorities on the carbohydrates. His book *Chemistry of the Carbohydrates*, published in 1948, became established as the standard reference text on the subject, and earned him wide recognition among students and specialists in a multitude of disciplines for which carbohydrates have relevance: organic chemistry, biochemistry, food and nutrition, medical aspects, and industrial utilisation.

Specialists in the carbohydrate field have also benefited from the organisational capabilities of Dr Pigman, who devoted many years of endeavour to development of the area through work with the Carbohydrate Division of the American Chemical Society and later with the International Society for Complex Carbohydrates, as well as through his own research papers, numbering more than 250, and his consistent efforts to bring research on the carbohydrates to the broader audience by means of review publications, monographs, symposia and lectures, and radio broadcasts. In 1959, he was honoured with the C. S. Hudson Award of the ACS Carbohydrate Division for his outstanding contributions to the field of carbohydrates.

Born in Chicago, Pigman initiated his higher education there at a junior college, but moved to Washington, D.C. in 1930 to enter George Washington University, where he completed his B.S. and M.S. degrees while working in the laboratory of Dr Horace S. Isbell at the National Bureau of Standards. He remained at the Bureau altogether until 1943, while completing his Ph.D. degree (1936) at the University of Maryland and, during 1938-1939, spending a period of research study at the University of Leipzig under a grant from the Lalor Foundation.

The physical-organic emphasis in Isbell's laboratory, concerned with precise analytical standards and procedures with carbohydrates, provided Pigman

with a rigorous experience of accurate analytical technique, especially polarimetry, and he made important contributions to our understanding of the optical rotational and tautomeric properties of sugars and their derivatives. From this basis there developed an extensive series of investigations on enzymology, with emphasis on the glycosidases, a subject that Pigman also pursued while he was in Germany. During the later part of his stay at the Bureau of Standards, he worked on a project for the production of industrial alcohol from wheat; alcohol was then a scarce commodity needed for the war effort.

In 1943, he left the government research service and worked for a time with the Corn Products Refining Co. in Argo, Illinois, where the major interest was starch technology and utilisation. In 1946 he joined the research staff at the Institute of Paper Chemistry in Appleton, Wisconsin, to investigate various aspects of wood chemistry. During this period he became involved, with R. M. Goepp, Jr, in the planning of the book *Chemistry of the Carbohydrates*, which was completed by Pigman following the death of Dr Goepp in an accident. Dr Pigman also served as a founder of the reference series *Advances in Carbohydrate Chemistry* that had its debut in 1945, and he was an editor of the first four volumes.

Together with the scientific recognition from his writings, Pigman also came into the public eye during the 'red spy' scares in 1948-1949, when he was called upon to testify before a federal grand jury in the indictment and subsequent perjury trial of Alger Hiss; accusations that Pigman was involved in a leak of government documents were proved to be untrue.

A substantial transition of emphasis toward applied biochemistry occurred for Pigman when, in 1949, he moved to the University of Alabama Medical Center in Birmingham, Alabama to become Associate Professor of biochemistry. A primary phase of his research there was concerned with the mechanism and control of dental caries, with particular emphasis on the role of carbohydrates in the incidence of tooth decay. He continued active work with the American Chemical Society in service to the Sugar (later Carbohydrate) Division in its efforts to improve exchange of information

and ideas between workers from a broad range of backgrounds. He became a member of the Institute of Dental Research and the American Society of Biochemists, and his research interests progressed to detailed studies on carbohydrates of animal fluid secretions, especially hyaluronic acid, mucins, and glycoproteins.

He edited a multi-authored volume *The Carbohydrates*, which appeared in 1957 and brought the earlier book up to date; a subsequent edition was later prepared in collaboration with myself. In 1960, Professor Pigman moved to New York City to become Chairman of the Biochemistry Department at New York Medical College, while still retaining research connections in Alabama. He received the medal of the French Biochemical Society in 1962 and the University of Milan Medal in 1964.

Administrative duties in the new post were more substantial, especially during the years 1963-1968 when he served as Dean of the Graduate School of Basic Sciences. Since he could make decisions rapidly and delegate responsibility effectively, he was still able to concentrate on research, especially on mucins and glycoproteins. With his research group of seasoned investigators, he probed especially the structures of the submaxillary mucins and, never being afraid to take a controversial stand, he took issue with some of the accepted dogmas of protein biosynthesis as they apply to the formation of glycoproteins.

Sensing a need for broader contact between carbohydrate specialists and experts in other areas, particularly with respect to the complex conjugates of carbohydrates involved in biomedical problems, Professor Pigman became a founder and driving force behind the International Society for Complex Carbohydrates. He served as President of that Society and, on September 30, 1977, was attending the last day of a highly successful meeting of the Society in Woods Hole, Massachusetts, when a sudden seizure took his life. The proceedings of that meeting are dedicated to his memory. At the time of his death, he was organising a symposium on glycoproteins that will be held in Anaheim, California during March, 1978.

Ward Pigman was a tall, striking figure, white-haired in his later years; in his youth he was athletic and a good

basketball player. A great lover of the arts, especially of modern painting, theatre, and ballet, he also found much enjoyment in travel; in particular he took pleasure in sharing these activities in his later life in the company of his second wife, Gladys. He heeded little the counsel of those friends who suggested that his driving life-style was scarcely a recipe for longevity, but he lived a full and rich life with never-flagging zest for new ideas and schemes, coupled with a broad imagination and flair for accomplishment. His achievements in exploring and expounding on the most widespread group of natural products, the carbohydrates, in all of their aspects, have left a permanent impression on scientific history.

In addition to his wife, Gladys, living at their home in Jamesburg, New Jersey, Professor Pigman is survived by a brother and a sister, his sons, John and James, and daughter Jean of his first marriage, and three grandchildren.

Derek Horton

H. E. Street

PROFESSOR H. E. STREET of the Department of Botany of the University of Leicester died on 4 December 1977, aged 64. The news of his death will sadden a wide range of scientists, in the U.K. and abroad, his academic colleagues and a large number of former students, research associates and many friends. Their collective sense of loss will be more acute in that the end was so unexpected.

Professor Street was in what was expected to be the last year of his active Professorship but it was known that he was planning for some more years of laboratory work in the field of his current interests. These interests fall into that area of plant physiology and of the study of growth which utilises aseptic conditions to culture erstwhile mature and quiescent tissue, to make it grow rapidly again, and whether from randomly proliferating explanted tissue or cells, to induce the development of new plants.

Professor Street's enthusiasm for this aspect of botanical work expressed itself, not only in a steady stream of research papers in the scholarly journals but also by his participation in symposia on the international level. At least as early as 1956 at Woodstock in Vermont, U.S.A. and in 1961 in Delhi, India and almost annually since then, Professor Street has been an invited guest and honoured contributor to such meetings. But more recently he lent his support to the organisation of such conferences, in part through the International Plant Tissue Culture Association which he helped to found. One such conference, widely attended at

Leicester in 1974, owed much of its success to his initiative and, in that case, he edited the resultant volume.

Throughout his career Professor Street actively fulfilled his desire to communicate science—to students and teachers whose understanding of basic biological science he cultivated and to a larger general audience who sought to comprehend the science and relate it to technology and society. He served a term as President of the Botanical Section of the British Association for the Advancement of Science, and always enjoyed demonstrating his work at scientific meetings such as those of the Society for Experimental Biology and, on occasion, at Royal Society Conversations.

In these various roles Professor Street was an inveterate traveller to botanical conferences whether they were large (like the last International Botanical Conference at Leningrad in 1975) or smaller (like the symposium on Plant Cell and Tissue Culture at Columbus, Ohio as recently as September 1977). At these gatherings his somewhat shaggy mane (whitening and thinning with the years) his cheerful laugh and the inevitable pipe (always being relit but never, it seemed, completely so) and his indefatigable attendance through the dullest sessions, which by his presence and questions he so often lightened, will be a memory that many will cherish.

Professor Street's most infectious quality was zest; an enthusiasm for the subject of botany that he always retained. But this emerged after he came to academic botany later than most. He trained first in pharmacy then obtained an external degree from London in 1939 with 1st class honours while still working at Nottingham. A Ph.D., at Birkbeck College, from London University was taken not only under the difficulties of part-time study while being employed, but also amid the disturbances of World War II, for his research had to be moved first to his home (anticipating the wartime fate that eventually overtook the laboratory in Central London) and then to Manchester.

Professor Street's early interests involved the nitrogen metabolism of plants, and he maintained close contact with this subject for some years, but later he concentrated on the areas that finally captured his interest and loyalty. This began when he turned to the aseptic culture and nutrition and the indefinite growth of isolated root tips in liquid, at Manchester University after the completion of his doctoral degree. He was awarded a D.Sc in 1953.

Later, at Swansea, where he was to serve for 13 years as Professor of Botany in the University of Wales, Professor Street transformed the

Botany Dept. of the University College of Swansea for he presided over the building of well equipped botanical laboratories and made them an internationally known centre for the study of growth. But he sought association with a larger group of biologists and so moved to the University of Leicester.

Though one feared that an increasing administrative responsibility (he became the first Chairman of a School of Biology there) would have dampened his research ardour, this did not occur for he maintained his enthusiasm to the end. He will be sorely missed, not only by his wife and three children to whom our sympathy is extended, but as a teacher, research worker and as a protagonist for botanical science wherever botanists and plant physiologists study the problems of growth and form.

F. C. Steward

Ana Conea

DR ANA CONEA died on 4 March, 1977 in the great Bucharest earthquake. She was a leading Romanian soil scientist who had achieved particular success in the fields of soil classification and systematics. The Bucharest earthquake claimed many lives and the loss of Dr Conea, aged only 54, was particularly tragic.

She was born on 20 July, 1922 and graduated in geography from Bucharest State University in 1945. In 1966 she received her doctors degree for her work on pedogenetical formations and Quaternary deposits in central and northern Dobrudja, and this formed the basis for the book *Formațiuni Cuaternare în Dobrogea* which was published in 1970 by Editura Academiei, Bucharest. She also produced Guidebook 10 of the Romanian Geological Institute which deals with the loess in Romania.

Dr Conea was an active member of the Internationale Quartärvereinigung (INQUA) Loess Commission and provided the Romanian data for the INQUA loess map of Europe which is one of the Commission's major undertakings. She was also a member of the Paleopedology Commission of the International Society of Soil Science and of the ECA/FAO Group for the 1:1,000,000 Soil Map of Europe. She published more than 90 papers during her 26 years work at the State Committee of Geology, the Geological Institute, and latterly at the Research Institute of Soil Science and Agrochemistry. She worked on the geography of Romanian soils, complex soil survey, paleopedology and paleogeography. Her main interests were in the fields of soil classification and syste-

matics and she was the main author of the new Romanian soil classification system.

Her work had a continental as well as a national significance and her death leaves the European community of soil scientists and loess investigators perceptibly impoverished.

I. J. Smalley

Arthur Erdélyi

ARTHUR ERDÉLYI, FRS, Professor of Mathematics in the University of Edinburgh, died suddenly at his home on 12 December 1977 at the age of 69.

Professor Erdélyi was a mathematician of immense talent and earned an international reputation for his research in many aspects of mathematical analysis.

He had, by any standards, a remarkable life. He lived in Eastern Europe until his early thirties when he was forced as a Jew to leave, in mortal danger, and seek refuge in Britain in 1939. Due largely to the good offices of the late Sir Edmund Whittaker, he was able to settle in Edinburgh and work in the university there. In the late 1940s he was attracted to California but fifteen years later he returned to the University of Edinburgh as Professor of Mathematics, a position he filled with great distinction until his death.

The fifteen years he spent at the California Institute of Technology constituted the period at which his mathematical talents were at their height. He had gone originally to examine the research notes of the late Professor Harry Bateman on Special Functions and subsequently led a team of analysts to produce an integrated record based on his work. The resulting five volumes of the "Bateman Project", three on the properties of Special Functions and two devoted to tables of Integral Transforms, will almost certainly be regarded as the final definitive treatment of this topic.

It is not, however, for this that Arthur Erdélyi would like to be mainly remembered. Important as the applications of Special Functions were and are, there was a danger of the subject degenerating into sterile manipulations. In addition, the advance of computer technology had made less important the detailed properties of the functions themselves. It was therefore appropriate that, having produced the complete survey of the subject, Arthur Erdélyi should turn his powerful analytical mind to other fields.

There were two topics in particular in which important developments occurred in the early 1950s and with which Erdélyi became deeply involved,

then and subsequently. The first was a systematic and rigorous approach to generalised functions, a topic envisaged by the pragmatic approaches of Heaviside and Dirac, and first given a proper framework by Schwartz. The second was the modern theory of singular perturbations, strained coordinates and matched asymptotic expansions in which mathematicians at the California Institute of Technology were particularly active. Erdélyi became known internationally as an expert in these fields although there were many other areas in which he worked actively and effectively.

From 1964 until his death, he was Professor of Mathematics at Edinburgh University. He returned to a department which had been dominated for over 50 years by the great names of Whittaker and Aitken and was to prove a worthy successor. The department flourished under his leadership, helped, as he was the first to acknowledge, by the cooperation of old and new colleagues alike.

Perhaps the most abiding memory of him which his mathematical colleagues will cherish was the remarkable breadth of his knowledge. He was able to talk with authority on almost any topic in mathematics and his commentaries were often laced with anecdotes and personal recollections and always given in impeccable English.

Outside his mathematics, he had a great love of music and of all things artistic. He was a man of ready wit and great personal charm. His many friends all over the world feel a sense of deep loss at his death but will ever recall with pleasure the privilege of having known him.

A. G. Mackie

Christopher Goetze

CHRISTOPHER GOETZE, Associate Professor of Geology at M.I.T., died on 21 November, 1977 at the age of 38. He received his training at Harvard, obtaining the AB in Physics in 1961 and PhD in Geophysics in 1969. His doctoral studies were carried out with Francis Birch on attenuation in salt mixtures.

During his eight years at M.I.T., Goetze and his associates became one of the world's leading groups in the study of the rheology of rocks. They extended the creep measurements of olivine to temperatures as high as 1700 °C and to stresses as low as 50 bars. These were very important developments, for it enabled the flow law of olivine—the most important constituent in the upper mantle—to be determined rather accurately for the first time at upper mantle conditions.

The mechanism of plastic flow of rocks at high temperature involves dislocation motion. Goetze and his associates pioneered techniques of dislocation identification. For example, they so improved the resolution using the transmission electron microscope that lattice planes in olivine or pyroxene could be directly imaged for the first time. They discovered that collapse of dislocation loops provided a measure of diffusion in olivine. They also discovered a simple way of decorating dislocations in olivine to make them visible optically. They showed for the first time a clear distinction between creep associated with dislocations and that associated with stress corrosion.

Goetze showed how dislocation theory combined with laboratory calibration experiments could be used to measure paleostresses in ancient deformed rocks. His establishment of a paleostress scale for quartz, olivine, and calcite gave structural geologists for the first time a measure of the stress active during the geological deformation of these minerals.

In 1971 Goetze suggested the use of ion thinning to expose microcracks in rocks for direct examination. This opened up a whole new field. Several groups now map cracks, using this technique, with such diverse applications as geothermal energy extraction, and earthquake prediction.

Goetze was a brilliant experimentalist with a keen understanding of the physics of deformation. His laboratory became recognised worldwide. A number of foreign scientists were attracted here for sabbatical visits. He was an invited lecturer at the Royal Society in London in 1977 and at the NATO Advanced Institute Summer School in Geophysics at Cargèse, Corsica, in the summer of 1976. He was also invited lecturer at a number of Penrose and Chapman conferences and was to have lectured at a conference on the physics of magmatic processes at Princeton in November 1977. During the last couple of years he had been able to extend the laboratory results to a number of major geophysical problems such as the stress near plate boundaries, the role of shear heating in the upper mantle, the role of volatiles and melt in upper mantle flow, and the mechanical history of certain meteorites.

A noted mountain climber, Goetze established new routes on Mount McKinley and in the Hayes range in Alaska, the Wind River Range in Wyoming, in British Columbia, and in Labrador. He cruised the waters of Newfoundland and Northern Labrador in a small sailboat and in a kayak for many years on vacations with his family.

W. F. Brace

announcements

Meetings

5-7 April, **Recent Advances in the Biochemistry of Cereals**, Bangor (Dr D. L. Laidman, Department of Biochemistry and Soil Science, University College of North Wales, Bangor Gwynedd, UK).

6 April, **Lead Pollution—Health Effects**, London (The Conservation Society, 168 Dora Road, London SW19, UK).

6-7 April, **Computers for Image-making**, Cardiff (British Universities Film Council, 81 Dean Street, London W1, UK).

12 April, **Burns and Drug Action**, London (Society for Drug Research, c/o Institute of Biology, 41 Queens Gate, London SW7, UK).

10-12 April, **EUCON—Energy Utilisation and Conservation in Industry**, London (Peter Mirrington, EUCON '78, Brintex Exhibitions Ltd, 178-202 Great Portland Street, London W1, UK).

15-17 April, **Field meeting on the Carboniferous of Cornwall**, Cornwall (Palaeontological Association, Dr C. T. Scrutton, Department of Geology, The University, Newcastle upon Tyne, UK).

17-18 April, **Drugs and Immune Responsiveness**, London (Mrs J. Kruger, Department of Pathology, Royal College of Surgeons of England, 35-43 Lincoln's Inn Fields, London WC2, UK).

17-20 April, **10th National Atomic and Molecular Physics Conference**, Egham (The Institute of Physics, 47 Belgrave Square, London SW1, UK).

17-21 April, **American Geophysical Union, Spring Meeting**, Miami (American Geophysical Union, 1909 K St, N.W. Washington, D.C. 20006).

25 April, **The Chemical Control of Plant and Fungal Growth**, Wye (*attendance limited*; The Secretary, ARC Unit, Wye College, Ashford, Kent, UK).

18-19 April, **Organofluorine Chemicals and their Industrial Applications**, Birmingham (Society of Chemical Industry, 14 Belgrave Square, London SW1, UK).

1-4 May, **DNA—Repair and Late Effects, Annual Meeting**, Israel (IGEGM. Institut. für Biologie, Forschungszentrum Seibersdorf, A-2444 Seibersdorf, Austria).

11 May, **Energy Storage**, Southampton (The Society of Chemical Industry, 14 Belgrave Square, London SW1, UK).

14-18 May, **69th Annual Meeting of the American Oil Chemists Society**, St Louis (American Oil Chemists' Society, 508 South Sixth St, Champaign, Illinois 61820).

16 May, **Cancer—Solutions within our Grasp**, London (The Marie Curie Memorial Foundation, 124 Sloane Street, London SW1, UK).

19 May, **The Nutrition Society—past, present and future, AGM**, London (The Nutrition Society, Chandos House, 2 Queen Anne Street, London W1, UK).

5-8 June, **12th Annual Conference on Trace Substances in Environmental Health**, Missouri (Dr D. D. Hemphill, 411 Clark Hall, University of Missouri-Columbia, Missouri 65201).

7-8 June, **Natural History of newly emerging and re-emerging Viral Zoonoses**, Munich (P. A. Bachmann, Director, WHO Collaborating Centre, Königinstr. 49, 8000 Munich 22, West Germany).

7-9 June, **The Mechanisms of Pain and Analgesic Compounds**, Baltimore (E. G. Bassett, Miles Laboratories, Inc., Elkhart, Indiana 46514).

8-10 June, **International Symposium on Chemical Toxicology of Food**, Milan (Nutrition Foundation of Italy, Via S. Pietro all-Orto 17, Milan, Italy).

12-16 June, **International Symposium on Biological Nitrogen Fixation**, Madison (W. H. Orme-Johnson, Department of Biochemistry, University of Wisconsin-Madison, Madison, Wisconsin 53706).

12-17 June, **The Large Scale Characteristics of the Galaxy**, Maryland (F. J. Kerr, Astronomy Program, University of Maryland, College Park, Maryland 20742).

12 June-25 August, **The Gordon Research Conferences—Frontiers of Science**, New Hampshire (Dr Alexander M. Cruickshank, Director Gordon Research Conferences, Colby-Sawyer College, New London, New Hampshire 03257).

13 June, **11th International Symposium on Osteoporosis**, Miami (Mrs Frieda Fagin, Continuing Medical Education, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, New York 10461).

13-16 June, **2nd International Symposium on Quantitative Mass Spectrometry in Life Sciences**, Gent (Prof. Dr A. De Leenheer, Laboratoria voor Medische Biochemie en Klinische

Analyse, De Pintelaan 135, b-9000 Gent, Belgium).

14-15 June, **31st Chemist Conference**, Scarborough (The British Independent Steel Producers Association, 5 Cromwell Road, London SW7, UK).

18-24 June, **Advanced Institute in Methods of Immunological Diagnosis**, Michigan (Noel R. Rose, Department of Immunology and Microbiology, Wayne State University School of Medicine, 540 East Canfield, Detroit, Michigan 48201).

19-21 June, **5th International Symposium on Mass Spectrometry in Biochemistry and Medicine**, Rimini (Dr Alberto Frigerio, Istituto di Ricerche Farmacologiche, "Mario Negri", Via Eritrea 62-20157 Milan, Italy).

22 June-6 July, **Progress and Problems in Cosmic Ray Astrophysics**, Sicily (M. M. Shapiro, Naval Research Laboratory, Washington, D.C. 20375).

25-30 June, **Cell Biology and Biochemistry of Leukocyte Function**, Israel (Prof. M. R. Quastel, The Sorka Medical Center, PO Box 151, Beer-Sheba, Israel).

26-28 June, **1978 Meeting of the Pacific Slope Biochemical Conference**, Santa Barbara (K. K. Tewari, Department of Molecular Biology and Biochemistry, University of California, Irvine, California 92717).

29-30 June, **5th Annual Conference of the Plasma Physics Group**, St Andrews (Institute of Physics, 47 Belgrave Square, London SW1, UK).

Reports and publications

Other countries—December

Australia: Commonwealth Scientific and Industrial Research Organization. Research Report of the Division of Applied Organic Chemistry, 1975/1976. Pp. 32. (East Melbourne, Victoria: CSIRO, 1977.) [2812]

Australian Journal of Zoology. Supplementary Series. No. 53: The Australian Genus *Brachyhesma* (Apoidea: Colletidae), Revised and Reviewed. By Elizabeth M. Exley. Pp. 54. No. 54: The Philotarsidae (Insecta: Psocoptera) of Australia. By I. W. B. Thornton and T. R. New. Pp. 62. (East Melbourne, Victoria: Editorial and Publications Service, CSIRO, PO Box 89, 1977.) [2812]

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Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Forest Research, 1976/1977. Pp. 77. (Canberra, ACT: CSIRO, 1977.) [2812]

Thirty-second Annual Report of the Council of the Queensland Institute of Medical Research to the Honourable the Minister for Health for the year ended June 30, 1977. Pp. 35. (Brisbane: Queensland Institute of Medical Research, 1977.) [2912]

Some Thoughts on Truth. By D. S. Kothari. (Anniversary Address, 1975.) Pp. 22. (New Delhi: Indian National Science Academy, Bahadur Shah Zafar Marg, 1977.) [3012]

UK & Ireland—January

- Ministry of Agriculture, Fisheries and Food. Agricultural Development and Advisory Service. Regional Agricultural Science Service Annual Report, 1976. Pp.viii+235. (London: HMSO, 1977.) £8 net. [31]
- Republic of Ireland. National Drugs Advisory Board Annual Report, 1976. Pp.28. (Dublin: National Drugs Advisory Board, 1977.) [31]
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- Department of Energy. Fuel Efficiency Booklet No. 12: Energy Management and Good Lighting Practices. Pp.12. (London: Department of Energy, Thames House South, Millbank, SW1, 1977.) [51]
- Central Water Planning Unit. Nitrate and Water Resources with particular reference to Groundwater. Pp.vii+64. (Reading: Central Water Planning Unit, Reading Bridge House, 1977.) [51]
- Towards a More Conservative Energy Policy. By Nigel Forman. MP. Pp.80. (London: Conservative Political Centre, 32 Smith Square, SW1, 1978.) £1.50. [61]
- Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 281, No. 981: Observations on the Recent History of Lough Neagh and Its Drainage Basin. By R. W. Battarbee. Pp.303-345+plates 1-3. (London: The Royal Society, 1978.) £4.90. [61]
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- Proceedings of the Royal Irish Academy. Section B: Biological, Geological and Chemical Science. Vol. 77, B, No. 10: The Craighillyharkly Granitic Complex Within the Tyrone Igneous Series. By N. S. Angus. Pp.181. 199. (Dublin: Royal Irish Academy, 1977.) £1.44. [91]
- Social Science Research Council. Co-operation in Pre-School Education. By Joyce S. Watt. Pp.90. (London: Social Science Research Council, 1 Temple Avenue, EC4, 1977. Available from School Government Publishing Co., Ltd., Darby House, Bletchley Road, Merstham, Redhill, Surrey.) £1.50. [121]
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- Advances in Behaviour Research and Therapy, Vol. 1, No. 1, 1977. Pp.1-56. Annual subscription: UK £22.72; Overseas £27.77. Single part £7. (Oxford and New York: Pergamon Press, 1977.) [171]
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- Science Museum Library. Science Library Bibliographical Series No. 806: Some Metallurgical and Technical Writings of the Musher Family. Pp.9. (London: Science Museum Library, 1977.) [181]
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- Report of the Interagency Committee on Recombinant DNA Research: International Activities, November 1977. Pp.iv+120. (Bethesda, Md.: US Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, 1977.) [161]
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- carbon and Heavy Minerals Investigation in East Asia. Pp.vii+182. (Bangkok, Thailand: UNDP Technical Support for Regional Offshore Prospecting in East Asia, United Nations ESCAP, 1977.) [191]
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